UNIVERSITÉ DU QUÉBEC À MONTRÉAL

IDENTIFICATION ET ÉTUDES DE FONCTION ET DE RÉGULATION DE GÈNES ASSOCIÉS À LA VERNALISATION ET À LA TRANSITION FLORALE CHEZ LE BLÉ HEXAPLOÏDE (*Triticum aestivum* L.)

THÈSE

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COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOCHIMIE

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TABLE DES MATIÈRES

LISTE DES FIGURES

LISTE DES TABLEAUX

LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

°C	Celsius grades
µ	micro
aa	amino acid
AAC	Agriculture et Agroalimentaire Canada
At	*Arabidopsis thaliana*
Bp	base pair
CA	cold acclimated
CCB	Commission canadienne du blé
CBF	*C-repeat binding factor*
ChIP	Chromatin ImmunoPrecipitation
cDNA	complementary deoxyribonucleic acid
COR	*Cold Regulated*
Cv	cultivar
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization
Fig.	Figure
FT	freezing tolerance
FW	fresh weight
g	gram
g	gravity speed
gDNA	genomic deoxyribonucleic acid
h	hour
H3	Histone 3
H3Ac	Histone 3 acetylation
H3K4me3	Histone 3 lysine 4 trimethylation

H3K27me3	Histone 3 lysine 27 trimethylation
IgG	immunoglobulin G
JA	Jasmonic acid
kDa	kilodalton
L	litre
m	milli
M	molar concentration (mol/litre)
MeJA	Methyl jasmonate
MS	mass spectra
mRNA	messenger ribonucleic acid
NCBI	National Center of Biotechnology Information
NA	non acclimated
NV	non vernalized
PAGE	polyacryloamide gel electrophoresis
PCR	polymerase chain reaction
pH	negative decaic logarithm of the proton concentration in mol per litre
PR	pathogenesis-related
QTL	quantitative trait loci
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RNA	ribonucleic acid
rpm	revolution per minute
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate poly*a*crylamide gel electrophoresis
TaIRI	*Triticum aestivum Ice Recrystallization Inhibition*
TaFT1	*Triticum aestivum FLOWERING LOCUS T like 1*
TaVRN1	*Triticum aestivum VERNALIZATION1*

TaVRN2	*Triticum aestivum VERNALIZATION2*
TaVRN3	*Triticum aestivum VERNALIZATION3*
TFs	transcription factors
V	vernalized
VRN	*VERNALIZATION*
WCOR	*Wheat Cold Regulated*
WCS	*Wheat Cold Specific*
WT	wild type

RÉSUMÉ

Les plantes sont des organismes sessiles, ce qui implique qu'elles doivent passer à travers tous les stades de leur développement au même endroit peu importe les conditions environnementales. La transition de la phase végétative (production de tiges et feuilles) à la phase reproductive (production de fleurs) est une étape cruciale de ce développement. Les facteurs environnementaux tels que la durée de l'ensoleillement, la température, les nutriments et la disponibilité en eau sont des déterminants importants de cette transition, et donc de la floraison. En zones tempérées, la majorité des espèces végétales ont développé des mécanismes d'adaptation leur permettant d'optimiser leur développement en fonction des conditions de température et d'ensoleillement. Des pertes agricoles importantes peuvent être encourues si ces conditions ne sont pas optimales. Le blé est une des espèces céréalières essentielles à cause de son utilisation quotidienne directe et indirecte dans la nourriture de la population humaine mondiale et des animaux. Cette céréale est composée essentiellement de cultivars d'hiver et de cultivars de printemps. Les cultivars d'hiver sont semés en automne et récoltés en début d'été, donc traversant tout l'automne et l'hiver avec le risque de pertes dues au gel. Les cultivars de printemps sont semés au printemps et récoltés en fin d'été avec le risque de pertes dues au gel d'automne et aux attaques d'insectes ravageurs plus abondants à ce moment. La floraison du blé d'hiver requiert une exposition prolongée aux températures basses, un processus appelé vernalisation. Chez le blé hexaploïde (*Triticum aestivum L.*), le processus de vernalisation est régi par une des voies génétiques qui impliquent trois gènes principaux qui codent pour des facteurs de transcription, *Triticum aestivum VERNALIZATION1* (*TaVRN1*), *Triticum aestivum VERNALIZATION2* (*TaVRN2*) et *Triticum aestivum FLOWERING LOCUS T like 1* (*TaFT1*), également appelé *TaVRN3*. *TaVRN1* et *TaVRN3* sont des activateurs de la floraison tandis que *TaVRN2* est un répresseur de la floraison. Afin de comprendre la fonction et la régulation de chacun de ces trois gènes dans la floraison du blé hexaploïde, j'ai utilisé une approche génomique (biopuce) combinée à des outils bioinformatiques (analyse de données et de séquences) et une approche de génétique moléculaire (régulation de l'expression génique, épigénétique, et mutagène).

Dans une première étude, j'ai testé si VRN2 peut agir comme un répresseur de la floraison chez une espèce de plante différente des céréales tempérées. Pour atteindre cet objectif, nous avons exprimé de façon constitutive chez *Arabidopsis thaliana* le gène du blé *TaVRN2*. Les plantes transgéniques obtenues n'ont montré aucune altération de leur morphologie, mais leur date de floraison a été considérablement retardée par rapport aux plantes contrôles. Ces résultats indiquent que TaVRN2, bien que n'ayant pas d'orthologue connu chez les *Brassicaceae*, agit comme un répresseur de la floraison chez ces espèces. Des études sur la tolérance au gel ont révélé que les plantes transgéniques ont une tolérance au gel plus élevée que

les plantes contrôles. Dans l'ensemble, ces données suggèrent que le gène *TaVRN2* pourrait moduler des voies de régulation qui régissent le temps de floraison et l'induction de la tolérance au gel.

Des études d'expression de la transcription indiquent que l'expression de *TaVRN-A1* et *TaFT-A1* chez le blé d'hiver est induite par la vernalisation. En utilisant la méthode d'immunoprécipitation sur la chromatine (IPCh ou ChIP) nous avons démontré que cette régulation à la hausse est associée à une augmentation du niveau de méthylation de l'histone-3-lysine-4-triméthylation (H3K4Me3) impliquée dans la régulation épigénétique alors qu'on ne note pas de changement du niveau de l'histone-3-lysine-27-triméthylation (H3K27Me3) à la région promotrice de *TaVRN-A1* et *TaFT1-A1*. Cependant pour les deux marqueurs, leur niveau d'expression est maintenu comparable à la région promotrice *TaVRN-B2*. H3K4me3 est un marqueur d'activation de la transcription alors que H3K27Me3 est un marqueur de répression de la transcription. Des résultats d'analyses de séquences de promoteur de chaque gène obtenus avec l'utilisation d'outils bioinformatiques révèlent la présence d'éléments *cis* ciblés par des facteurs de transcription communs chez les espèces de plante qui exigent la vernalisation pour fleurir. Ces données suggèrent l'implication de ces éléments *cis* ciblés durant la vernalisation. Ces promoteurs possèdent également des éléments de réponse au Polycomb et au Trithorax qui lient les groupes de protéines Polycomb et Trithorax, pour maintenir les états de transcription réprimé ou actif des gènes de développement importants. L'ensemble de ces données indique que la transition de la floraison induite par la vernalisation chez le blé est régulée de façon épigénétique et est médiée par la méthylation des histones au niveau des promoteurs de *TaVRN1*, *TaFT1* et T*aVRN2*. Ceci peut représenter une partie de la mémoire cellulaire de vernalisation chez le blé.

Afin d'étudier l'impact de l'absence du gène *VRN1* sur des gènes associés à la floraison, nous avons utilisé une approche génomique basée sur une analyse du transcriptome des plantes d'un mutant *mvp-1* (*maintained vegetative phase*) contenant une délétion de gènes incluant *VRN1*. Les résultats de cette analyse indiquent que cette délétion conduit à la régulation de 368 gènes. Parmi les gènes hautement régulés, on compte ceux associés à la réponse aux pathogènes (PR) et aux jasmonates. Ces résultats suggérent que cette délétion dans les plantes du mutant *mvp-1* causant l'absence de floraison est associée à l'activation de la réponse moléculaire du mécanisme de défense modulée par la biosynthèse de l'hormone méthyl jasmonate (MeJA). Pour confirmer l'implication du MeJA dans la floraison, nous avons mesuré la teneur en MeJA dans les plantes homozygotes du mutant *mvp-1* et des plantes de type sauvage. Le contenu en MeJA était six fois plus élevé dans les plantes du mutant *mvp-1* en comparaison du contenu du MeJA dans les plantes de type sauvage. Un traitement avec 150 µM de MeJA sur du blé de printemps hexaploïde (cv Manitou) montre un retard de floraison de deux semaines et une croissance réduite des plantes traitées. Ce retard dans la floraison était associé à la répression significative du gène *Triticum aestivum FLOWERING LOCUS T like 1* (*TaFT1*) supportant ainsi le rôle possible du MeJA dans le contrôle de la floraison.

Les résultats obtenus dans le cadre de ces travaux de doctorat montrent l'importance du rôle des gènes *VRN1, VRN2* et *VRN3* ainsi que de l'hormone MeJA dans la mise en place des mécanismes d'adaptation pour mieux synchroniser la floraison et le développement du blé face aux changements environnementaux. En perspective, cette étude offre des avenues prometteuses afin d'améliorer des stratégies agricoles et la productivité céréalière.

Mots clé: Acclimatation au froid; Biopuce; Blé; Épigénétique; Facteurs de transcription; Floraison; Immunoprécipitation de chromatine; Mutant; Photopériode; Transgénique; Tolérance au gel; Vernalisation.

CHAPITRE I

PROBLÉMATIQUE

Le blé constitue la plus importante source d'énergie dans l'alimentation. La culture du blé occupe 17 % des terres cultivables (en 2002, 210 millions d'hectares *vs.* 147 millions pour le riz et 139 millions pour le maïs) (Gill *et al.*, 2004). Plus de 60% de l'apport en calories et en protéines de l'alimentation journalière proviennent du blé, du riz et du maïs. Dans les pays nordiques comme le Canada qui est un grand producteur de blé, les basses températures entraînent une baisse de productivité agricole qui cause des pertes économiques considérables. Selon Agriculture et Agroalimentaire Canada (AAC) et la Commission Canadienne du Blé (CCB), le blé représente la principale culture produite au Canada et la première source de gains d'exportation parmi tous les produits agricoles du pays. Dans son rapport annuel publié pour la campagne agricole 2009-2010, la CCB a rapporté des recettes brutes d'exportation de 5,2 milliards de dollars. Selon AAC, le Canada fait partie des trois plus grands exportateurs de blé au monde et il est le premier producteur mondial de blé de mouture riche en protéines. En 2009, le Canada a produit plus de 26 millions de tonnes de blé. De plus, la recherche agricole au Canada a permis de développer des variétés de blé dur riches en gluten, très prisées par les fabricants de pâtes alimentaires du Canada et d'autres pays. Le blé roux de printemps de l'ouest canadien est reconnu comme un blé haut de gamme pour la fabrication du pain.

Dans des régions tempérées ou froides comme le Canada, les plantes doivent s'adapter à des périodes relativement longues de froid et une période d'ensoleillement écourtée. Ces plantes ont acquis la compétence de faire face à une longue période de froid et de mettre en place une programmation de leur développement afin d'acquérir ou d'accélérer leur phase reproductible durant cette période de froid. Ce processus "d'acquisition ou de l'accélération de la capacité de fleurir suite à une exposition prolongée au froid" est nommé la vernalisation (Chouard, 1960). Le processus d'acclimatation au froid est induit rapidement durant les premiers moments d'exposition au froid des plantes alors que le processus de vernalisation se met en place durant une longue période d'exposition au froid des plantes. Chez certaines

plantes, en plus du froid, leur processus de transition florale est régulé par le temps d'exposition à la lumière ou la photopériode.

Dans ces régions à hautes latitudes, les périodes chaudes de l'année (printemps et l'été) sont associées avec l'allongement des journées (longue photopériode (PP)) alors que les périodes défavorables de froid sont associées à des journées écourtées (courte photopériode) (automne et hiver). Il est devenu évident que la durée de photopériode influence l'expression de nombreux gènes de régulation, y compris les gènes qui contrôlent le développement de la transition florale. Le processus de floraison est une étape fondamentale de la reproduction des plantes. Chez les plantes à fleurs, la transition entre les phases végétative et reproductive dépend à la fois de signaux endogènes (l'âge et l'état physiologique de la plante), des facteurs génétiques (l'expression des gènes qui induisent la production des organes floraux au niveau du méristème) et des conditions environnementales favorables, la longueur des jours et une température suffisamment clémente. Ainsi, la mise en place de ce processus de transition doit se faire au moment opportun et dans les meilleures conditions. Les basses températures n'induisent pas seulement la tolérance au gel via l'acclimatation au froid, mais elles affectent aussi la programmation de plusieurs processus développementaux comme la vernalisation et la floraison. Chez *Arabidopsis thaliana*, où l'on retrouve les mêmes phénomènes, la floraison repose sur un réseau complexe de gènes, parmi lesquels il y a un répresseur central de la transition florale, le *FLOWERING LOCUS C* (*FLC*) (Henderson et Dean, 2004). Les études moléculaires ont montré que, chez les variétés sensibles à la vernalisation, l'exposition aux basses températures des plantules ou des graines en cours de germination conduit à la répression du gène *FLC* jusqu'à la fin du cycle de reproduction. De plus, l'intensité de la répression augmente progressivement avec le temps passé au froid, et cette mémoire des conditions hivernales détermine la précocité de la floraison au retour des conditions favorables au printemps (Sheldon *et al.*, 2000).

Chez les plantes céréalières d'hiver, le temps de floraison est retardé jusqu'à ce que les conditions favorables de croissance arrivent. Ce retard est modulé par les basses températures via le processus de vernalisation. Sur la base des allèles *VERNALIZATION1*(*VRN1*) et *VERNALIZATION2* (*VRN2*) et de leurs interactions, trois cultivars majeurs ont été définis chez le blé et l'orge: le cultivar d'hiver, qui a besoin de vernalisation, le cultivar de printemps, qui n'a pas besoin de vernalisation, et le cultivar facultatif, qui n'a pas non plus besoin de la vernalisation, mais qui est fortement sensible à la photopériode. Le cultivar de printemps a au moins un allèle dominant *VRN1*, c'est à dire, la transition vers la phase de reproduction ne peut être réprimé par la vernalisation. Le cultivar facultatif a la constitution allélique *VRN2VRN2 / VRN1VRN1* ce qui signifie que l'allèle *VRN1* n'est pas réprimé par *VRN2* (et donc le cultivar facultatif n'a pas besoin de vernalisation), mais il peut être réprimé par d'autres gènes, par exemple, des facteurs de transcription activés par la photopériode ce qui fait que le cultivar facultatif est très sensible à la photopériode. Par conséquent, le cultivar facultatif reste en mode croissance végétative, sauf s'il est stimulé pour fleurir en période de jours longs. Le cultivar d'hiver a la constitution allélique *VRN2_/vrn1vrn1* ce qui signifie qu'il a un besoin de vernalisation pour réguler à la baisse l'expression de l'allèle *VRN2* en vue de dé-réprimer l'allèle *VRN1* (Szücs *et al.*, 2007 ; von Zitzewitz *et al.*, 2005). Chez les variétés qui portent des allèles dominantes de *FT1* et de *VRN1*, la floraison est induite sans traitement préalable à la vernalisation (Yan *et al.*, 2006). Chez *Arabidopsis thaliana*, FT interagit avec FD, un facteur de transcription appartenant à la famille «basic leucine zipper». FD active des gènes cibles incluant ceux appartenant à la famille de facteurs transcription MADS-box qui sont reliés à *VRN1*; *FRUITFUL* et *APETALLA1* (Corbesier *et al.*, 2007). Récemment, il a été démontré chez le riz, que Hd3a/FT interagit avec les protéines 14-3-3 dans les cellules apicales des pousses, ce complexe est transporté dans le noyau pour se lier au facteur de transcription FD1, un homologue FD d'*Arabidopsis thaliana*. Le complexe ainsi formé, induit la transcription de *OsMADS15* l'homologue de *APETALA1* chez *Arabidopsis thaliana* et

homologue *VRN1*chez le blé ce qui conduit à la floraison (Taoka *et al.*, 2011). Chez le blé, la protéine FT1 peut aussi interagir avec la protéine FD-like 2 (TaFDL2), qui peut, à son tour, se lier au promoteur de *VRN1; a*insi, chez les céréales, *FT1* peut activer l'expression de *VRN1* dans les feuilles et au sommet des bourgeons grâce à des interactions avec les protéines de type FD (Li et Dubcovsky, 2008). L'activation de *VRN1* par *FT1* peut également se produire dans les variétés qui ne possèdent pas *VRN2*, où l'induction rapide des deux gènes *FT1* et *VRN1* se produit lorsque les plantes sont exposées à des photopériodes de jours longs (Yan *et al.*, 2006). Bien que l'activation de *FT1* peut induire l'expression de *VRN1* chez certains génotypes, la pertinence que la vernalisation induise la floraison chez ces génotypes n'est pas claire. Chez les variétés de blé d'hiver semées en automne, *VRN1* est induit par le froid durant l'automne lorsque les journées sont de courte durée (Danyluk *et al.*, 2003 ; von Zitzewitz *et al.*, 2005) et *FT1* n'est pas exprimé (Hemming *et al.*, 2008). De même, la vernalisation induit l'expression de *VRN1* dans les graines germées dans l'obscurité où *FT1* n'est pas exprimé (Sasani *et al.*, 2009). Ces résultats suggèrent que l'induction de *VRN1* par le froid via *FT1* est peu probable. De même, l'activation de *VRN1* en période de longue photopériode pourrait permettre à certaines variétés de fleurir, même si les plantes ne subissent pas de vernalisation. L'activation de *VRN1* en période de jours longs pourrait aussi être importante après la transition vers développement du système reproducteur car les longues journées peuvent augmenter l'expression de *VRN1* dans les feuilles après l'initiation de l'inflorescence (Sasani *et al.*, 2009). Cela peut se produire par du mécanisme de rétroaction positive permettant à *FT1* d'accroître l'expression de *VRN1*, qui active encore *FT1*. Ce qui pourrait renforcer la réponse de la floraison en période de jours longs une fois que l'inflorescence et le développement commencent.

Le blé d'hiver semé en automne, qui exige la vernalisation pour fleurir, peut s'acclimater aux basses températures et est par la suite capable de survivre dans des conditions de gel durant l'hiver. Ce processus est connu comme étant l'acclimatation

au froid (Galiba *et al.*, 1995). Plus les plantes sont exposés à de basses températures, plus elles augmentent leur tolérance au gel. Cela continue jusqu'au moment où le traitement par le froid pourrait avoir un impact défavorable sur la mise en place du processus de floraison (le point de saturation de la vernalisation), puis la tolérance au gel commence à diminuer (Prášil, Prášilová et Pánková, 2004). Ainsi, il semble que l'acclimatation au froid et la vernalisation sont interconnectées. L'activation du gène *VRN1* peut causer la diminution graduelle de la tolérance au gel qui commence quand les plants sont suffisamment vernalisés. Cette hypothèse est validée par des études génétiques montrant que les allèles dominants de *VRN1* réduisent la tolérance au gel (Fowler *et al.*, 1996). Par exemple, une comparaison des lignées quasi-isogéniques qui diffèrent que par le génotype de *VRN1* a montré que les variétés avec des allèles dominants de *VRN1*, qui ne nécessitent pas la vernalisation pour fleurir, ont une capacité très réduite à s'acclimater au froid par rapport aux lignées de type sauvage possédant un allèle récessif de *vrn1* qui exigent la vernalisation pour fleurir (Koemel *et al.*, 2004). À l'heure actuelle, il n'est pas clair si cela est une conséquence directe de l'induction de *VRN1* ou une conséquence indirecte, due à l'effet de *VRN1* sur d'autres gènes ou sur le développement des plantes en soi. Cependant, il est connu que le gène *VRN2* dont la présence de son allèle dominant *VRN2* et la présence l'allèle récessif de *VRN1* (*Vrn-2_/vrn-1vrn-1*) sont les marqueurs essentiels du blé d'hiver (Szucs *et al.*, 2007 ; von Zitzewitz *et al.*, 2005). Les plantes appartenant à famille des *Triticeae* comme le blé (*Triticum*), l'orge (*Hordeum*) et le seigle (*Secale*) sont des céréales d'importance économique. Ces céréales forment un groupe homogène et un système colinéaire de leurs génomes (à savoir génomes, A, B et D chez le blé cultivé *Triticum aestivum*, génome H chez *Hordeum vulgare* et du génome R chez *Secale cereale*). Cela signifie que les gènes présents dans un génome d'un *Triticeae* ont leurs homologues situés à la même position chromosomique dans les autres (à part quelque exceptions déclenchées par des délétions, des duplications, des insertions ou des translocations). En conséquence de ce fait important, les résultats obtenus sur un membre des *Triticeae* peuvent être extrapolés aux autres.

Étant un grand producteur et exportateur de blé, le Canada n'est pas à l'abri de pertes économiques liées à son climat très rigoureux qui peut causer une baisse importante de sa productivité agricole. Afin de développer des stratégies pour réduire les risques de dommages sur les cultures du blé durant la transition de leur phase végétative à leur phase reproductive qui pourraient être causés par le climat rigoureux du Canada, une meilleure compréhension du processus de floraison est requise pour augmenter la productivité agricole du blé et ainsi réduire les pertes économiques. Pour ce faire, l'identification et l'étude de fonctions et de régulations des gènes associés à la transition florale et à la vernalisation demeure essentielle.

CHAPITRE II

REVUE DE LITTÉRATURE

2.1 LA FLORAISON

Les plantes nous fournissent l'oxygène, la nourriture, des médicaments, des vêtements, des matériaux d'habitation et en plus, elles sont belles et agrémentent notre environnement de leurs couleurs et leurs odeurs. À l'instar de toutes les espèces, les plantes assurent leur survie en se reproduisant. Elles contrôlent leur processus de floraison en tenant compte de certains facteurs abiotiques et chimiques notamment, la température, la lumière, l'eau et les nutriments etc., et de certains facteurs biotiques (champignons, virus et bactéries etc.). Cette prise en compte des différents facteurs permet aux plantes de s'assurer que la floraison se fasse au moment opportun et dans les meilleures conditions. La caractérisation de mutants allèliques et naturels chez la plante modèle, *Arabidopsis thaliana (At)* a permis d'identifier quatre voies de régulation de la floraison (Blazquez, 2000). Ces voies identifiées sont : la voie autonome dont les principaux gènes sont des loci de floraison (*FCA*, *FY*, *FLD (FLOWERING LOCUS D)* et *FVE)*; la voie hormonale (ou gibberilline) avec comme principaux gènes *LEAFY* (*LFY)* et *SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC-1)*; la voie de la photopériode dont les gènes majeurs sont *CONSTANS* (*CO)*, *FLOWERING LOCUS T* (*FT)* et *SOC* et la voie de la vernalisation dont les gènes majeurs sont *Vernalization Insensitive 3* (*VIN 3*) et *FLC* (Amasino, 2004 ; Blazquez, 2000).

L'analyse de mutants affectés dans la voie de vernalisation chez *Arabidopsis thaliana* a conduit à l'identification de plusieurs facteurs spécifiquement impliqués dans la répression de *FLC* par le froid (Henderson et Dean, 2004). Le gène *VERNALIZATION INSENSITIVE 3* (*VIN 3*) code pour une protéine à homéodomaine retrouvée dans plusieurs complexes de remodelage de la chromatine (Sung et Amasino, 2004). Son expression est induite par la vernalisation et coïncide avec

l'inhibition du gène *FLC*. Chez le mutant *vin3*, l'expression de *FLC* demeure inchangée après traitement par le froid. Des expériences d'immunoprécipitation de la chromatine ont par ailleurs montré que la répression de *FLC* par *VIN3* implique des modifications profondes de la chromatine au niveau du locus cible, qui passe d'un état « euchromatinien », caractérisé par une hyperacétylation des histones H3 et H4 ainsi qu'une triméthylation de la lysine 4 de l'histone H3 (H3K4me3), à un état « hétérochromatinien », marqué par une désacétylation des histones H3 et H4 et une méthylation des lysines 9 et 27 de l'histone H3 (H3K9me2 et H3K27me3) (He et Amasino, 2005). Il faut cependant noter que la méthylation de l'ADN, une marque de régulation épigénétique est étroitement associée à l'hétérochromatine chez *Arabidopsis* (Lippman *et al.*, 2004). Chez *Arabidopsis thaliana*, des études ont montré que la méthylation de l'ADN ne semble intervenir à aucun moment dans la répression de *FLC* (Bastow *et al.*, 2004 ; Sung et Amasino, 2004). La modification majeure de l'ADN est la méthylation de la cytosine, qui est généralement la marque d'une chromatine transcriptionnellement silencieuse chez les vertébrés (Bird, 2002). Quant aux histones, elles sont les pièces maîtresses de la compaction de l'ADN en chromatine et jouent un rôle majeur dans la régulation des fonctions du génome. Elles sont les cibles de multiples modifications post-traductionnelles qui apportent une information épigénétique. L'ensemble de ces modifications constituerait un « code histone », permettant d'associer à chaque combinaison de modifications un état particulier de la chromatine. L'épigénétique est définie comme « l'étude des modifications de l'expression des gènes qui sont transmissibles lors de la mitose et/ou la méiose, mais ne découlant pas de modifications dans la séquence de l'ADN » (Wollfe et Matzke, 1999). Au niveau des histones les modifications majeures post-traductionnelles qui ont été décrites sont l'acétylation, la phosphorylation, la méthylation et l'ubiquitinylation (Jenuwein et Allis, 2001 ; Turner, 2002). La mise en place du programme épigénétique est cruciale au cours du développement, et sa stabilité est essentielle pour le maintien des fonctions de chaque type cellulaire au cours de la vie d'un organisme (Li, 2002). Au cours de l'évolution, le développement

du système endogène des plantes a permis de pouvoir mesurer l'exposition au froid pendant une longue période de froid : un tel système aide les plantes à distinguer entre le printemps et les alternances thermiques qui ont lieu à l'automne (Sung et Amasino, 2005).

Dans les pays nordiques comme le Canada, grand producteur de blé, où le climat est très rigoureux, les basses températures entraînent des pertes économiques suite à une baisse de productivité agricole. L'acclimatation au froid augmente le niveau de tolérance au gel et peut induire le processus de vernalisation lorsque l'exposition au froid est prolongée. Chez les céréales comme le blé hexaploïde, les bases moléculaires de la vernalisation sont peu connues comparativement à *A. thaliana.* Ceci est principalement dû au fait que le génotype du blé qui implique cette réponse est associée à un caractère récessif (*vrn-A1*), à la complexité génétique et à la difficulté d'obtenir des mutants. D'où l'importance d'étudier les gènes responsables de la transition florale et de la vernalisation chez le blé. Ci-dessous sont illustrés les phénotypes de plants de blé en phase végétative et reproductible (Figure 1).

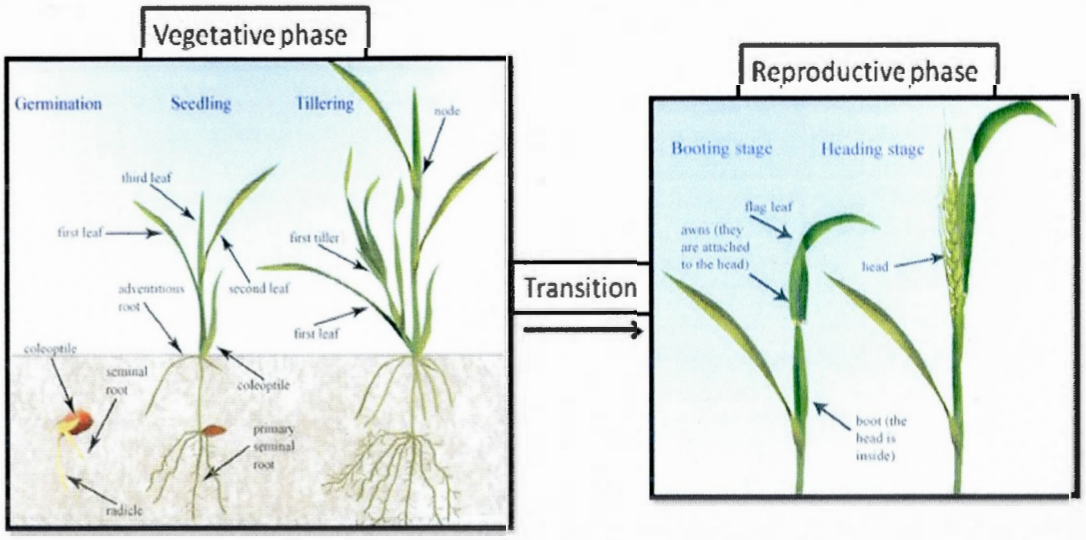

Figure 2.1 Photos de plants de blé tirées du site web NEMOSE.com et modifiées pour illustrer la distinction phénotypique des plants en phase végétative et en phase reproductive

2.1.1 L'ACCLIMATATION AU FROID ET LA TOLÉRANCE AU GEL

Les plantes qui poussent dans les régions où il se produit régulièrement de longues périodes de froid et / ou de gel au cours de l'année ont adapté leur cycle de vie face à ces conditions de croissance défavorables. Généralement ces plantes annuelles et / ou biennales survivent en période de phase végétative lorsque leurs organes végétatifs présentent un niveau suffisamment élevé de résistance au gel. La résistance au gel est définie comme étant la capacité des plantes à survivre aux impacts du gel (températures inférieures à zéro) et est habituellement correspondante à des valeurs de température létale (TL_{50}, lorsque 50% des plantes meurent à cette température). La tolérance au gel est inductible chez de nombreuses espèces de plantes y compris les *Triticeae* (blé, orge et seigle). La distinction entre des plantes sensibles au gel et des plantes résistantes au gel réside dans le fait que les plantes résistantes au gel peuvent induire un niveau élevé de tolérance au gel lorsqu'elles sont exposées durant une période suffisamment longue aux basses températures, mais au-dessus du point de congélation. Par contre les plantes sensibles au gel ne peuvent pas induire ce niveau de tolérance au gel dans ces mêmes conditions. Le seigle non acclimaté, par exemple, est tué par congélation à environ -5 °C, mais après un période d'exposition à des températures basses supérieures à zéro, le seigle peut même survivre à une congélation à environ -30 °C (Thomashow, 1999). En outre, il faut généralement une exposition assez longue aux basses températures pour atteindre le maximum de résistance au gel chez les cultivars tolérants. Par exemple, les plantes très tolérantes de blé d'hiver Mironovskaya 808 atteignent la tolérance maximale au gel après un traitement aux basses températures de 4 semaines à 3 °C (Prášil, Prášilová et Pánková, 2004). Ce type de réponse, à savoir, l'acquisition progressive de la tolérance au froid par des températures au-dessus du point de congélation (10° C à 0° C) chez une plante, est appelé processus d'acclimatation au froid.

En outre, la capacité de la plante d'induire un niveau élevé de résistance au gel est dépendante de son stade de développement. Les espèces appartenant à la tribu des *Triticeae* sont capables d'induire un niveau élevé de résistance seulement quand elles sont dans la phase végétative. Au cours de l'acclimatation au froid, de nombreux changements physiologiques et biochimiques se produisent dans les cellules végétales. Ces changements sont destinés à la compensation de la perte d'eau dans le cytoplasme cellulaire exposé aux basses températures ceci afin d'éviter la congélation intracellulaire. Le potentiel hydrique du cytoplasme de la cellule diminue en raison de l'accumulation de molécules tels que, les sucres (glucose, fructose, saccharose, raffinose), les alcools de sucre (mannitol, sorbitol), les composés ammonium quaternaire (glycine bétaïne, bétaïne alanine), les polyamines (spermine, spermidine, putrescine), et l'immino acide proline. Dans les membranes, la porportion en acides gras insaturés augmente afin de conserver une fluidité suffisante des membranes et des complexes protéiques transmembranaires (Guy, 1990 ; Sakai et Larcher, 1985). En conséquence, de nombreuses protéines spécifiques au froid sont synthétisées. Ces protéines peuvent agir soit en tant que molécules qui éliminent les espèces réactives de l'oxygène (ROS) qui sont produites en quantités relativement élevées avec le froid. D'autres molécules de synthèse d'acides gras insaturés peuvent agir comme chaperonnes, c'est-à-dire peuvent protéger d'autres protéines et structures à l'intérieur des membranaires contre des changements structuraux défavorables. Un groupe important de ces protéines de structure sont les protéines COR / LEA (cold-regulated/late embryogenesis abundant) qui s'accumulent dans le cytoplasme des cellules, les chloroplastes et le noyau dans les conditions associées à la déshydratation cellulaire, des conditions de stress environnementaux (sécheresse, salinité accrue, l'évaporation accrue, le froid, gel) et des conditions physiologiques de la maturation des embryons et la dessiccation dans les derniers stades de l'embryogenèse (d'où le nom de protéines LEA). En conséquence des modifications biochimiques décrites ci-dessus, les exigences sur le métabolisme énergétique des plantes augmentent lors du processus d'acclimatation au froid. Par conséquent, les *Triticeae* sont généralement

capables d'induire des niveaux plus élevés de résistance au gel en période de jours longs que dans des conditions de photopériode de jours courts quand ils sont dans leur phase végétative, car ils sont capables de synthétiser ces molécules en plus de les assimiler dans ces conditions (Limin *et al.*, 2007).

2.1.2 LES PROTÉINES COR / LEA INDUITES PAR LE FROID

Les protéines COR / LEA constituent un grand groupe de protéines structurales d'importance qui s'accumulent durant la déshydratation cellulaire. Ils sont généralement très hydrophiles et peuvent protéger d'autres protéines de structures membranaires contre la perte de leur enveloppe d'hydratation. La perte de l'eau est associée à des changements structurels et fonctionnels défavorables des biomolécules. Les protéines LEA sont généralement divisées en trois grands sous-groupes en fonction leurs séquences caractéristiques uniques: LEA3-L1, LEA3-L2 et LEA3-L3 (Cattivelli *et al.*, 2002 ; Ingram et Bartels, 1996 ; NDong *et al.*, 2002). Chez le blé et l'orge, les gènes *Lea* induits par le froid comprennent de nombreux gènes Lea II - déhydrines; chez l'orge, l'induction par le froid a été décrite pour Dhn5 et Dhn8 (Choi, Rodriguez et Close, 2002); chez le blé tendre, l'induction par le froid est caractérisée par l'expression d'une famille de gènes *Wcs120* comprenant *Wcs200*, *Wcs180*, *Wcs66*, *Wcs120*, *Wcor825* et *Wcor726* gènes (Sarhan, Ouellet et Vazquez-Tello, 1997) et une famille de gènes Wcor410, comprenant *Wcor410a*, *Wcor410b* et les gènes *Wcor410c* (Danyluk *et al.*, 1994 ; Danyluk *et al.*, 1998). Certains de ces gènes *Cor / Lea* induits par le froid peuvent être considérés comme des marqueurs de la résistance au gel, c'est-à-dire, le niveau d'accumulation de leurs produits correspond quantitativement au niveau de tolérance au gel chez différents cultivars de blé et d'orge. Par exemple chez le blé, les protéines de la famille WCS120 sont des marqueurs de tolérance (Houde, Dhindsa et Sarhan, 1992). Un effet positif de l'expression de certains gènes *Cor / Lea* du blé sur l'amélioration de la résistance au

gel a également été montré par des études de transformation transgénique chez d'autres espèces de plantes (NDong *et al.*, 2002 ; Shimamura *et al.*, 2006). L'expression des gènes *Cor / Lea* est modulée par plusieurs voies de régulation qui peut être essentiellement divisées en ABA-dépendantes et ABA-indépendantes. Les gènes *Cor / Lea* dont l'expression est principalement régulée par l'ABA contiennent plusieurs éléments de régulation *ABRE* dans leurs régions promotrices qui servent comme site de liaison pour les facteurs de transcription bZIP. Les éléments de régulation ABRE possèdent deux fragments: TACGTCC (le G-box) et GGCCGCG (GC-motif) (Allagulova *et al.*, 2003 ; Thomashow, 1999 ; Yamaguchi-Shinozaki et Shinozaki, 2005).

2.1.3 LES GÈNES *CBF* ET LES LOCI DE RÉSISTANCE AU GEL (FR LOCI)

L'une des plus importantes voies de régulation *ABA*-indépendante est la voie de signalisation *CBF* (C-repeat binding factor). Chez *Arabidopsis thaliana*, quatre facteurs de transcription CBF (CBF1 - CBF4) ont été identifiés. Trois d'entre eux (CBF1/DREB1B, CBF2/DREB1C et CBF3/DREB1A) sont induits par le froid et disposés en tandem sur le chromosome 4, tandis que CBF4/DREB1D est induit par la sécheresse. Les facteurs de transcription CBF se lient au domaine d'éléments de régulation CRT / DRE / LTRE dans les régions promotrices de leurs gènes effecteurs (par exemple, de nombreux gènes *Cor / Lea*). Les éléments CRT (C-repeat) / DRE (dehydration response element) / LTRE (low-temperature responsive element) (CRT / DRE / LTRE) contiennent une séquence caractéristique GCCGAC qui sert de site de liaison du domaine AP2 des facteurs de transcription CBF (Yamaguchi-Shinozaki et Shinozaki, 2005). L'expression de facteurs de transcription CBF chez *Arabidopsis thaliana* est contrôlée en partie par le facteur de transcription ICE1 (inducteur de l'expression de *CBF 1*; un facteur de transcription avec un domaine bHLH liant l'ADN) qui se lie aux éléments MYC dans les régions promotrices des gènes *CBF*

(Chinnusamy *et al.*, 2003). L'expression du gène *CBF3* est principalement régulée par ICE1, alors que les gènes *CBF1* et *CBF2* ne sont influencés que très légèrement. La majorité des gènes *CBF* chez les *Triticeae* ont été localisés sur le bras long des chromosomes homologues du groupe 5 au locus *Fr-2* (Choi, Rodriguez et Close, 2002 ; Francia *et al.*, 2004), l'un des deux QTL (Quantitative Trait Locus) majeur pour la tolérance au gel qui est aussi l'un des deux QTL majeurs de régulation de l'expression du gène *Cor14b* (Vágújfalvi *et al.*, 2000 ; Vágújfalvi *et al.*, 2003). Il est également devenu évident que les gènes *CBF* chez les *Triticeae* sont plus nombreux [l'orge -20 *CBFs* (Skinner *et al.*, 2005), le blé en grain, *T. monococcum* - 13 *CBFs*, (Miller, Galiba et Dubcovsky, 2006) et le blé tendre, *T. aestivum* jusqu'à 25 *CBFs* ont été proposés (Badawi *et al.*, 2007)]. Chez l'orge et *T. monococcum*, les *CBFs* ont été divisés en trois sous-groupes phylogénétiques distincts (Miller, Galiba et Dubcovsky, 2006 ; Skinner *et al.*, 2005). Chez le *T. aestivum*, les *CBFs* ont été divisés en dix sous-groupes (Badawi *et al.*, 2007) dont six d'entre eux ont été caractérisés comme spécifiques aux *Pooideae*. Il est également intéressant de noter que chez le blé et l'orge, les membres des sous-groupes, qui ont des similarités de séquences sont étroitement liés au *CBF1* d'*Arabidopsis thaliana*. Les gènes *CBF3*, sont situés dans des régions chromosomiques autres que les loci *Fr-2*. Cette grande diversité des *CBFs* chez les *Triticeae* peut être une conséquence de leur adaptation au climat tempéré.

Des études d'expression ont montré que cinq des sous-groupes spécifiques des *Pooideae* affichent de hauts niveaux d'expression constitutive et inductible par l'acclimatation au froid chez le cultivar de blé d'hiver Norstar par rapport au cultivar de blé de printemps Manitou (Badawi *et al.*, 2007). Ces niveaux élevés d'expression constitutive et inductible présents sont probablement associés à un caractère héréditaire des cultivars d'hiver. Ceci pourrait être la base de l'augmentation des capacités de tolérance au gel des cultivars d'hiver par rapport à ceux du printemps. Des différences quantitatives d'expression de gènes *CBFs* de différents cultivars

résistants au gel ont également été observées par d'autres chercheurs chez le blé (Miller, Galiba et Dubcovsky, 2006 ; Vágújfalvi *et al.*, 2005) et chez l'orge (Stockinger *et al.*, 2007). Ces auteurs ont également confirmé des différences d'expression constitutive de certains *CBFs* au niveau du locus *Fr-2*. Ils ont également découvert que l'expression quantitative de certains *CBFs* chez l'orge dépend de la photopériode. D'autres études ont porté sur l'affinité de protéines CBF à différents motifs CRT présents dans les promoteurs des gènes *Cor*. Il a été démontré qu'il y a une interaction de *HvCBF1* avec la séquence (G / a) (C / t) CGAC présente dans les promoteurs des gènes *Cor* (Xue, 2002). L'activation des gènes *Cor*, *Wdhn13* et *Wrab17* du blé par WCBF2 du blé a été démontrée chez des plantes transgéniques du tabac (Takumi, Shimamura et Kobayashi, 2008). De plus, il a été démontré que l'affinité de liaison des domaines AP2 des CBFs est également dépendante de la température; l'auteur a observé que la baisse de température conduit à une meilleure affinité du HvCBF2 aux éléments de motif de base GCCGAC du CRT / DRE / LTRE (Xue, 2003). Ainsi, il peut être proposé qu'en fonction de la température, l'affinité de liaison des CBFs peut exercer un autre niveau de régulation de l'expression génique des gènes *Cor*.

Le locus *Fr-2* et le locus majeur de résistance au gel nommé *Fr-1* a été cartographié sur les bras longs des groupes de chromosomes 5 homologues chez les génomes des *Triticeae*. Le locus *Fr-1* est éloigné du locus *Fr-2*, mais lié étroitement avec le locus *VRN1*. Ainsi, chez l'orge, le locus *Fr-H1* est étroitement lié au locus *VRN-H1*, et chez le blé hexaploïde, le locus *Fr-A1* est lié au locus *VRN-A1* (intervalle de 2 cM, (Galiba *et al.*, 1995), le locus *Fr-B1* est lié au locus *VRN-B1* (intervalle de 40 cM, (Tóth *et al.*, 2003)) et le locus *Fr-D1* est liée au locus *VRN-D1* (Intervalle de 10 cM, (Snape *et al.*, 1997)). Les deux loci *Fr* sur le bras long du chromosome 5 ont également été décrits comme étant les QTLs affectant l'expression du gène *Cor14b* chez le blé (Vágújfalvi *et al.*, 2000). Une étude d'expression réalisée chez *T. aestivum* a suggéré que le locus *FR-1* peut contrôler l'expression des gènes *Wcbf2* et

donc la régulation à la hausse de l'expression des gènes *Cor / Lea* en aval au moins partiellement par la voie de signalisation dépendante des CBF (Kobayashi *et al.*, 2005). Chez le blé, il est suggéré que l'allèle *Vrn-A1* des cultivars de printemps est associé à un allèle de type *Fr-A1* et inversement, l'allèle de type *vrn-A1* est étroitement lié à l'allèle *Fr-A1* des cultivars d'hiver. Dans les génomes B et D, il a été proposée que la situation est analogue. Chez le blé tendre, *Vrn-A1* est la copie du *VRN1* qui possède le niveau d'expression le plus élevé, il est également proposé que la copie *Fr-A1* du gène *Fr-1* a un effet majeur sur l'expression des gènes induits par le froid par rapport aux autres copies de gènes *Fr-B1* et *Fr-D1*. Des études utilisant des lignées quasi isogéniques (near isogenic lines NILs) chez le blé tendre ont montré que *Vrn-B1* NIL (NIL) a une plus grande tolérance au gel que *Vrn A1* NIL. Il est probable que *Vrn B1* NIL possède un allèle *Fr-A1* dominant de type d'hiver tandis que le *Vrn-A1* NIL possède un allèle *FR-A1* de type printemps (Kobayashi *et al.*, 2005). Dans le génome A, le locus *Fr-A1* serait si étroitement lié au locus *Vrn- A1* qu'ils peuvent être identiques et que le produit du gène *Vrn-A1* pourrait réguler l'expression des gènes *CBFs* (Miller, Galiba et Dubcovsky, 2006). Toutefois, les cultivars de blé possédant un allèle dominant *Vrn-D1* ou un allèle récessif *vrn-D1* peuvent ne présenter aucune différence significative de tolérance au gel (Ishibashi *et al.*, 2007). Cependant, dans le génome D, les loci *Vrn-D1* et *Fr-D1* ne seraient pas étroitement liés entre eux, et par conséquent, un niveau similaire de tolérance au gel acquis peut être trouvé chez les cultivars de blé d'hiver et de printemps différents seulement par une constitution allélique de *VRN-D1*. De plus des études ont montré que le locus *Fr* décrit à l'origine comme lieu de position du locus *Fr-B1* (Tóth *et al.*, 2003) est orthologue du locus *Fr-A2* chez le blé. En conséquent, son nom a été remplacé par *Fr-B2* (McIntosh *et al.*, 2004). De façon similaire, chez l'orge le locus *FR-H1* de l'orge est étroitement lié au locus *VRN-H1* et il a été montré que le QTL (Quantitative Trait Locus) de tolérance au gel sur le chromosome 5 HL ne peut pas être clairement séparé du QTL pour le besoin de vernalisation chez une population cartographiée d'orge issue d'un croisement entre le cultivar Nure (hiver) et le cultivar

Tremois (printemps) (Francia *et al.*, 2004). Les études portant sur les relations entre l'activité des produits du gène *VRN1* et l'expression des gènes *Cor / Lea* ont clairement montré que l'induction de la formation de méristèmes floraux et l'induction de l'expression du gène *VRN1* entraine l'expression à la baisse des gènes *Cor / Lea* (Danyluk *et al.*, 2003 ; Fowler *et al.*, 2001 ; Kane *et al.*, 2005 ; Kobayashi *et al.*, 2005 ; Kume *et al.*, 2005). De plus, il a été rapporté que le locus *VRN-H1/Fr-H1* affecte l'expression de plusieurs gènes CBF au locus *Fr-H2* dans une population cartographiée : Nure × Tremois de l'orge (Stockinger *et al.*, 2007). Les différents allèles *VRN1* affectent la capacité à maintenir un niveau suffisant de tolérance au gel en condition d'acclimatation au froid chez le blé d'hiver (Prášil, Prášilová et Pánková, 2004). Cependant, les interactions entre l'activité de VRN1 et l'expression des gènes *Cor / Lea* et le développement de tolérance au gel n'ont pas encore été précisément élucidées.

2.2 LA VERNALISATION

La vernalisation a été rigoureusement définie comme étant "l'acquisition ou l'accélération de la capacité de fleurir par un traitement prolongé au froid" (Chouard, 1960). Le développement de la transition vers la phase de reproduction, est indiqué chez les *Triticeae* par une formation de double crête sur le méristème apical, et est habituellement accompagnée par une baisse significative dans cette capacité de résistance au gel (Fowler *et al.*, 2001 ; Fowler *et al.*, 1996). Les plantes qui nécessitent une vernalisation ont besoin d'une exposition au froid suffisamment longue avant d'initier leur programme de transition de la phase végétative à la phase de reproduction. Les plantes en phase végétative peuvent induire un niveau assez élevé de tolérance au gel, tandis que les mêmes plantes dans la phase de reproduction ne le peuvent pas du aux organes génitaux (fleurs) qui sont généralement plus sensibles au froid (Sakai et Larcher, 1985). La vernalisation fonctionne donc comme

un important mécanisme de contrôle empêchant le développement des organes floraux qui moins tolérant au froid en début de transition de la phase reproductive. Au cours de vernalisation, des changements importants se produisent au niveau physiologique, biochimique et moléculaire.

2.2.1 LES GÈNES DE VERNALISATION

Chez le blé et l'orge, l'existence de trois loci de vernalisation a été démontrée. Ces loci ont été nommés *VRN1*, *VRN2* et *VRN-3*. Tous les loci de vernalisation codent pour des facteurs de transcription qui affectent la transcription de d'autres gènes.

Le locus *VRN1* code pour le gène majeur de la vernalisation qui contrôle la transition florale. Si le gène est actif, c'est à dire, le produit du gène est présent dans le noyau, de profonds changements dans l'expression de nombreux facteurs de transcription sont notés, ce qui conduit éventuellement à l'induction de la formation de méristèmes floraux. Le gène *VRN1* peut être présent dans le génome des céréales sous deux types d'allèles : répressible et irrépressible. L'allèle irrépressible *VRN1* est dominant, à savoir, s'il est présent dans le génome, il cause un effet majeur sur développement de la plante, indépendamment de d'autres allèles. L'allèle répressible *VRN1* peut être inactivé par la liaison de certains facteurs de transcription soit à la boite CArG-box dans la région du promoteur (Yan *et al.*, 2004), ou à une région spécifique 436 pb «une région critique de la vernalisation» dans le premier intron (Fu *et al.*, 2005). Il a été proposé que la boîte CArG-box dans le promoteur peut être un site potentiel de liaison d'un facteur de transcription MADS-box (Yan *et al.*, 2004). En revanche, la «région critique de la vernalisation» dans le premier intron permet de former quatre sites *Dof*, des cibles potentielles de facteurs de transcription avec un motif de liaison à ADN en doigt de zinc (von Zitzewitz *et al.*, 2005). L'allèle dominant de *VRN1* irrépressible possède une délétion dans ces régions critiques (Fu

et al., 2005). Ainsi, les répresseurs ne peuvent pas se lier à eux. Outre cette variabilité décrite ci-dessus, il a été montré que certains cultivars de printemps d'orge contiennent une insertion d'un élément transposable dans une région de l'intron 1, en amont de la région critique de la vernalisation (Cockram *et al.*, 2007). De manière analogue, une insertion d'un transposon a été signalée dans la région promotrice chez le gène *VRN-B3* (Yan *et al.*, 2006). Ainsi, les insertions de transposons semblent présenter un autre type de variation allèlique dans les loci *VRN*.

Le gène *VRN1* code pour un facteur de transcription appartenant à un MADS-box de la famille de l'*AP1*. Le même gène a également été nommé chez l'orge *HvBM5A* (*H. vulgare*) (von Zitzewitz *et al.*, 2005), le gène *TmAP1* chez le blé diploïde (*T. monococcum*) (Yan *et al.*, 2003) et les gènes *WAP1* (Murai *et al.*, 2003) et *TaVRT-1* (Danyluk *et al.*, 2003) chez le blé hexaploïde (*T. aestivum*). Le gène *VRN1* est situé sur le bras long du chromosome 5. Chez le génome de l'orge, il n'y a qu'un seul gène *VRN1* situé sur 5HL; ce gène est décrit comme *VRN-H1*. De manière analogue, chez le blé diploïde (*T. monococcum*), le seul gène *VRN1* est situé sur $5A^{m}L$ et décrit comme *VRN-*A^{m}*1*. Dans le blé hexaploïde (*T. aestivum*), il y a trois gènes *VRN1* décrits comme *VRN-A1* sur 5AL, *VRN-B1* sur 5BL et *VRN-D1* sur 5DL, respectivement. Alors que l'allèle dominant *Vrn-A1* réduit complètement le besoin de vernalisation chez le blé hexaploïde, les cultivars possédant seulement les allèles dominants *Vrn-B1* ou *Vrn-D1* présentent généralement certains besoins résiduels de vernalisation. Cette hypothèse a déjà été expliquée par une différence dans la transcription: la transcription de l'allèle dominant *Vrn- A1* apparaît plus tôt et en plus grande quantité dans les tissus des feuilles que le niveau de transcription des allèles dominants *Vrn-B1* et *Vrn-D1* au cours du développement des plantes (Loukoianov *et al.*, 2005). Les gènes *VRN1* chez les *Triticeae* sont étroitement liées aux gènes MADS-box de la sous-famille AP1/SQUA d'*Arabidopsis*, en particulier les gènes

impliqués à l'identité des méristèmes floraux *AP1* (*APETALA1*), *CAL* (*CAULIFLOWER*) et *FUL* (*FRUITFULL*) (Laurie *et al.*, 2004 ; Yan *et al.*, 2003).

Le locus *VRN2* est situé sur le chromosome 4HL chez l'orge. Toutefois, dans le génome A du blé, la région du chromosome 4AL contenant le locus *VRN-2* a été transférée à $5A^{m}L$ (Cattivelli *et al.*, 2002 ; Yan *et al.*, 2004b). Ainsi, le locus *VRN2* localisé au bras long du chromosome $5A^{m}L$ de *T. monococcum*. Le locus *VRN2* de l'orge se compose de trois gènes étroitement liés *ZCCT-Ha*, *ZCCT-Hb* et *ZCCT-Hc* (Dubcovsky, Chen et Yan, 2005). Chez *T. monococcum*, deux gènes étroitement liés nommé, *ZCCT-1* et *ZCCT-2* ont été identifiés au locus *VRN2* (Yan *et al.*, 2004b). *ZCCT-Ha* dans l'orge et *ZCCT-1* dans *T. monococcum* ont été signalés pour être des candidats probables pour les gènes *VRN2* (Dubcovsky, Chen et Yan, 2005 ; Yan *et al.*, 2004b). Tous ces gènes codent pour des protéines à doigts de zinc, CONSTANS, CONSTANS-like, et TOC (ZCCT), des facteurs de transcription qui sont régulés à la baisse par la vernalisation. Les facteurs de transcription ZCCT contiennent deux importants domaines de liaison C_2H_2 (2 cystéine 2 histidine) un domaine en doigt de zinc, qui peut se lier à la fois à de l'ADN et à des protéines et qui est codé par le premier exon, et le domaine CCT, qui contrôle la localisation nucléaire des facteurs de transcription (Robson *et al.*, 2001) et qui se lie à la boîte CCAAT un facteur de liaison *Cis* qui assure la médiation des interactions entre les protéines CONSTANS-like et l'ADN (Ben-Naim *et al.*, 2006). Le domaine CCT est codé par le second exon. Le gène *VRN2* peut également être présent en allèle dominant (fonctionnelle) et récessif (mutation ponctuelle entrainant une perte de fonction : *VRN2a* ou suppression complète : *vnr-2b*). Les mutations perte de fonction dans *ZCCT-1* sont associés à une substitution d'un seul acide aminé conservé soit une arginine par un tryptophane en position 35 du domaine CCT chez *T. monococcum* (Yan *et al.*, 2004b) et *T. aestivum* (Diallo *et al.*, 2010).

Le gène *VRN2* qui agit comme un répresseur central du gène *VRN1* et dont l'expression est régulée à la baisse par la vernalisation n'a pas d'orthologue chez *Arabidopsis thaliana*. Récemment nos travaux ont démontré qu'une surexpression du gène *VRN-B2* du blé hexaploïde chez *Arabidopsis thaliana* entraine un retard de floraison comparativement aux plantes contrôles (Diallo *et al.*, 2010). Chez *A. thaliana*, le répresseur central de la floraison est *FLC* qui code un facteur de transcription MADS-box appartenant à une sous-classe particulière, n'est pas identifié chez les plantes herbacées. Par analogie chez les *Triticeae*, le gène *VRN2* un répresseur de la floraison qui code pour un facteur de transcription ZCCT, n'a pas été encore identifié sur la voie de la réponse à la vernalisation chez *Arabidopsis* (Kane *et al.*, 2005). En outre, les domaines CCT à partir des protéines ZCCT des céréales appartiennent à un sous-groupe qui n'est pas présent chez *Arabidopsis* (Yan *et al.*, 2004b). Ces observations soutiennent la thèse selon laquelle, la régulation des voies de signalisation de la vernalisation chez *Arabidopsis* et les céréales ont évolué de manière indépendante.

La relation entre les gènes *VRN1* et *VRN2* est épistatique, c'est à dire, si un allèle dominant *VRN1* est présent dans le génome, sa présence se traduit par une plante de printemps indépendamment de la constitution allélique au locus *VRN2* (épistasie dominante de *VRN1* sur *VRN2*). Il a été suggéré par certains auteurs (Loukoianov *et al.*, 2005 ; Trevaskis *et al.*, 2006) que l'allèle dominant de *VRN1* pourrait agir comme un répresseur de l'allèle dominant de *VRN2*, à savoir, si un allèle dominant *VRN1* est présent dans le génome, il pourrait réguler négativement l'expression de *VRN2*. La plupart des *Triticeae* sauvages appartiennent aux cultivars d'hiver. Il semble peu probable qu'une exigence de vernalisation se soit développée indépendamment au même locus chez tous les *Triticeae*. Ainsi, il pourrait être proposé que chez les cultivars d'hiver leurs allèles récessifs *VRN1* sont ancestraux, et l'allèle dominant *VRN1* des cultivars de printemps a évolué de façon indépendante comme une perte de fonction de l'allèle (perte de l'exigence vernalisation) par des

mutations dans ses sites de régulation à la boîte CArG de son promoteur et la «région critique de la vernalisation» dans le premier intron (Yan *et al.*, 2003).

Le locus *VRN-3* code pour un facteur de transcription orthologue à *FT* chez *Arabidopsis thaliana* (*FLOWERING LOCUS T*) (Yan *et al.*, 2006) et il est situé sur le chromosome 7HS chez l'orge et 7BS chez le blé, où il était autrefois décrit comme locus *VRN-B4*. Les gènes candidats ont été nommés *HvFT1* chez l'orge et *TaFT1*chez blé. L'allèle dominant améliore la floraison via une augmentation de l'expression du gène *VRN1*. Chez l'orge, l'allèle dominant a une délétion dans son premier intron par rapport à l'allèle récessif, alors que chez le blé, l'allèle dominant *Vrn-3* possède une insertion d'un rétro-élément dans le promoteur à la différence de l'allèle récessif. Les allèles dominants de blé et de l'orge permettent d'améliorer la floraison en période de jours longs comme agit *FT* chez *A. thaliana* et il est proposé que les gènes *FT* sont placés sous le contrôle du locus *Ppd-1* (Kosová *et al.*, 2008).

Étant donné qu'aucune variation allélique du locus *VRN-3* n'a été observée dans la plupart des accessions cultivées du blé et de l'orge, un modèle à deux gènes épistatiques de vernalisation comprenant seulement les gènes *VRN1* et *VRN2* a été proposé (Szücs *et al.*, 2007 ; Yan *et al.*, 2003). Le gène *VRN2* est un répresseur de *VRN1*, c'est à dire qu'il inhibe l'expression du produit du gène *VRN1*. Dans ce modèle, le gène *VRN1* agit comme le gène majeur du développement c'est-à-dire permettant la transition de la phase végétative à la phase de reproduction des plantes. Les cultivars portant au moins un allèle dominant de *VRN1* n'ont pas du tout besoin de vernalisation. Si les cultivars ne portent que des allèles récessifs de *VRN1*, ces allèles peuvent être réprimés par le produit du gène *VRN2* (Dubcovsky *et al.*, 2006 ; Fu *et al.*, 2005). Le gène *VRN2* peut aussi être présent sous deux types d'allèles dominante (répresseur fonctionnel) et récessif (répresseur absent ou non fonctionnel). Il a été démontré que l'expression du gène *VRN2* est diminuée par la vernalisation (Yan *et al.*, 2004b) et la durée de la photopériode (Diallo *et al.*, 2010). La fonction de *VRN2*

en tant que répresseur de *VRN1* a été proposée sur la base d'une expérience de l'interférence à l'ARN (RNAi): cela a consisté en une insertion d'un segment complémentaire de l'ARN du gène ZCCT1 chez le cultivar de blé d'hiver hexaploïde *Jagger*, ce qui a abouti à la régulation à la hausse du gène *VRN1* et à la réduction de l'exigence de vernalisation (Yan *et al.*, 2004b). Ainsi, les cultivars d'hiver qui ne contiennent que des allèles récessifs de *VRN1* et au moins un allèle dominant de *VRN2* ont une exigence vernalisation. Plus tard, il a été montré que ce modèle n'est valable que dans des conditions de jours longs, tandis que les conditions de jours courts conduisent à la régulation négative de l'expression *VRN2* indépendamment de la température qui n'aboutira pas à la régulation à la hausse du gène *VRN1* chez les génotypes homozygotes récessifs *VRN1VRN1* (Dubcovsky *et al.*, 2006 ; Trevaskis *et al.*, 2006). Par conséquent, l'existence d'au moins un autre répresseur *VRN1*, qui peut réprimer son expression en période de jours courts a été postulé. Des travaux dans notre laboratoire ont démontré que ce répresseur peut être *Triticum aestivum Vegetative to Reproductive Transition* (*TaVRT-2)* un gène qui code pour un facteur de transcription appartenant à la famille des MADS-box comme *VRN1* qui peut se fixer sur la boîte CArG-box du promoteur de *VRN1* (Kane *et al.*, 2007 ; Kane *et al.*, 2005).

Voici ci-dessous la liste de gènes caractérisés chez le blé et l'orge (Table I).

Tableau 2.1 **La liste des loci de vernalisation et leurs gènes candidats**

Species	Locus	Gene - transcription factor	Location	Reference
H. vulgare	*VRN-H1* (formerly *Sh 2*)	*HvBM5A* – MADS-box	5HL	Von Zitzewitz *et al.* (2005)
	VRN-H2 (formerly *Sh*)	*ZCCT-Ha, ZCCT-Hb, ZCCT-Hc* – zinc-finger TF with CCT domain	4HL	Dubcovsky *et al.* (2005)
	VRN-H3 (formerly *Sh 3*)	*HvFT* - orthologue of *FT* in *A. thaliana*	7HS	Yan *et al.* (2006)
T. monococcum	*VRN-A^m1*	*TmAP1* – MADS box	5A^mL	Yan *et al.* (2003)
	VRN-A^m2	*ZCCT-1, ZCCT-2* (zinc-finger and CCT TF)	5A^mL	Yan *et al.* (2004b)
T. aestivum	*VRN-A1* (formerly *VRN1*)	*WAP1*- MADS-box	5AL	Murai *et al.* (2003)
	VRN-B1 (formerly *VRN2*)	*TaVRT-1*- MADS-box	5BL	Danyluk *et al.* (2003)
	VRN-D1 (formerly *VRN3*)		5DL	
	VRN-B3 (formerly *VRN-B4*)	*TaFT* - orthologue of *FT* in *A. thaliana*	7BS	Yan *et al.* (2006)

Chez le blé, *VRN1/TaVRN1* et *VRN2/TaVRN2* déterminent le type de cultivar et la floraison. En outre, le facteur de transcription MADS-box *Triticum aestivum Vegetatif to Reproductive Transition 2* (*TaVRT-2*) est également associé à la réponse de la vernalisation d'une manière similaire à *TaVRN2*. En utilisant des essais d'expression transitoire chez le tabac (*Nicotiana benthamiana*), nous avons montré que TaVRT-2 agit comme un répresseur de la transcription de *TaVRN1*. TaVRT-2 lie le motif CArG-box dans le promoteur *TaVRN1* et réprime son activité in vivo. En revanche, TaVRN2 ne lie pas le promoteur *TaVRN1* et n'a pas d'effet direct sur son activité, mais il peut augmenter l'effet de la répression de *TaVRT-2*. Cela suggère qu'un complexe répresseur régule l'expression de *TaVRN1*. Chez le blé d'hiver, les transcrits de *TaVRT-2*, *VRN2* et *VRN1* s'accumulent dans le méristème apical et les jeunes feuilles. Chez le blé de printemps non vernalisé cultivé sous une photopériode de jours courts, TaVRT-2 s'accumule et un retard de la floraison est observé, ce qui suggère que *TaVRT-2* est régulé de manière indépendante par la photopériode et par les basses températures. Ces observations suggèrent que TaVRT-2, en liaison avec VRN2, réprime la transcription de VRN1. Le gène *TaVRT-2* code pour une protéine de 226 acides aminés appartenant à la famille des facteurs de transcription MADS-box. Une recherche des bases de données publiques révèle des domaines conservés dans la protéine TaVRT-2 et la présence d'une structure MIKC (M, domaine MADS; I, intervenant région; K, boîte de K; C, domaine C-terminal). Une analyse phylogénétique classe TaVRT-2 dans le clade StMADS11 un groupe de facteurs de transcription associés à la répression de la transition de l'apex de la tige de l'état végétatif à la phase de reproduction (Kane *et al.*, 2007 ; Kane *et al.*, 2005).

2.2.2 LA MÉMOIRE DES PLANTES

Lorsque les cellules de blé vernalisé sont utilisées pour régénérer des plantes par culture de tissus, les plantes qui en résultent ne nécessitent pas la vernalisation pour fleurir (Marcinska, Dubert et Biesaga-Koscielniak, 1995). De même, les cellules du blé peuvent être vernalisées pendant la culture des tissus pour donner naissance à des plantes qui fleurissent rapidement sans qu'on soit obligé de vernaliser les plantules (Whelan et Schaalje, 1992). De plus, des graines en maturation peuvent être exposées à un froid prolongé et ensuite autorisées à subir le processus normal de déshydratation des graines et leur germination peut donner naissance à des plantules qui fleurissent sans vernalisation (Gregory et Purvis, 1936). Ces observations suggèrent que les céréales conservent une mémoire cellulaire de vernalisation. L'idée que les plantes conservent une mémoire de vernalisation est conforme à la façon dont la vernalisation favorise la floraison: lorsque les graines en germination sont exposées à de longues périodes de froid, il n'y a pas de signe visible de développement floral à la fin de vernalisation, mais les plantes subissent rapidement le développement floral lorsque placées à des températures de croissance favorables après vernalisation (Flood et Halloran, 1984 ; Purvis, 1934 ; Sasani *et al.*, 2009). Ainsi, la vernalisation est retenue et elle s'exerce au cours du développement ultérieur des plantules (Chouard, 1960). Après la floraison des plantes, la mémoire de vernalisation est réinitialisée dans les graines pour permettre la réponse à la vernalisation de se reproduire dans la génération suivante.

2.3 LA PHOTOPÉRIODE

2.3.1 LOCI SENSIBLES À LA PHOTOPÉRIODE ET GÈNES ASSOCIÉS QUI SONT IMPLIQUÉS DANS LA TRANSITION À LA FLORAISON

Certains processus de développement dans les chez plantes sont initiés lorsque la photopériode (durée du jour) est plus longue ou moins longue qu'une certaine durée appelée «durée critique». La photopériode critique n'est pas la même dans tous les cas, elle dépend de l'espèce végétale et du processus de développement qu'elle régule. Selon les effets de la photopériode sur l'induction de la floraison, les plantes peuvent être divisées en trois grands groupes: des plantes de jours longs (Long Day), des plantes de jours courts (Short Day) et des plantes de jours neutres. Des plantes de jours longs fleurissent lorsque la longueur du jour est plus longue qu'une certaine photopériode critique tandis que les plantes de jours courts fleurissent lorsque la longueur du jour est inférieure à une photopériode critique. Les plantes à jours neutres sont insensibles à la photopériode, c'est à dire, qu'elles fleurissent indépendamment de la longueur du jour. Pour les plantes sensibles à la photopériode l'implication des phytochromes et photo-récepteurs est essentielle.

Les céréales appartenant aux *Triticeae* sont sensibles à la photopériode. La transition à la floraison est induite durant les jours longs. Chez l'orge, les gènes majeurs associés à la photopériode qui contrôlent la transition florale sont situés à deux loci *Ppd* (photopériode) *Ppd -H1* et *Ppd -H2* (Laurie, 1997). Le locus *Ppd -H1* se trouve sur 2HS et est un inducteur principal de la floraison en période de jours longs (Karsai *et al.*, 1997 ; Laurie *et al.*, 1994). Le locus *Ppd -H2* se trouve sur 1HL et est un répresseur principal de la floraison en période de jours courts (Laurie *et al.*, 1995). Chez d'autres plantes, comme la plante modèle *Arabidopsis thaliana* et chez la pomme de terre, des gènes comme *CO-like* (*CONSTANS-like*) qui sont régulés par la photopériode contrôlent la transition vers la floraison (González-Schain et Suárez-

López, 2008 ; Putterill *et al.*, 1995). Neuf gènes *CO-like*, *HvCO1* à *HvCO9*, ont été identifiés chez l'orge (Griffiths *et al.*, 2003), mais ils ne sont pas situés au niveau des loci *Ppd* dans le génome de l'orge. Des études ont rapporté l'existence d'un mutant du blé tendre cv. Ciano 67 insensible à la photopériode qui porte un allèle *Ppd-D1a* qui a une délétion de 2 kbp en amont de la région codante du gène *PRR* qui est exprimé sous les deux photopériodes de jours longs et de jours courts (Beales *et al.*, 2007). Par conséquent, ce mutant fleurit indépendamment de la longueur du jour, probablement via une induction positive de *TaFT1* (*VRN-B3*), un régulateur positif majeur de l'expression de *VRN1* qui est normalement réprimé en condition de photopériode de jours courts. *HvFT3*, un gène qui appartient à la famille *HvFT* a été proposé comme gène candidat pour le locus VRN-3 et qui est impliquée dans la régulation de la floraison en période de jours courts, peut être un gène candidat pour le locus *Ppd-H2* (Faure *et al.*, 2007). Il est également très probable que d'autres facteurs de transcription HvFT (de la famille des gènes *HvFT* comprennent cinq membres *HvFT1* à *HvFT5* qui codent pour des protéines avec un domaine de liaison phosphatidyléthanolamine) sont impliqués dans la régulation photopériodique de la floraison. Il a été démontré par de nombreux auteurs que l'expression de gènes *VRN* est influencée par la photopériode (Dubcovsky *et al.*, 2006 ; Karsai *et al.*, 2005). On a découvert que l'expression du gène *VRN2* est régulée à la baisse en période de jours courts (Diallo *et al.*, 2010 ; Dubcovsky *et al.*, 2006). Toutefois, sa régulation à la baisse n'aboutit pas à la régulation positive de l'expression de *VRN1*.

Des études dans notre laboratoire ont montré l'existence d'un répresseur de *VRN1* nommé le gène *TaVRT-2* du blé et son orthologue, *HvVRT-2* chez l'orge est régulé à la hausse en période de jours courts et réprimé en période de jours longs chez l'orge cv. Dicktoo (Kane *et al.*, 2005) D'autres études nous ont permis de démontrer que TaVRT-2 peut se lier à la fois à la boîte CArG dans le promoteur du *VRN1* et à la protéine VRN2 (Kane *et al.*, 2007 ; Kane *et al.*, 2005). Ainsi, le modèle de régulation de *VRN1* commence à devenir plus complexe et il semble évident que les deux

phénomènes, la vernalisation et la photopériode affectent la répression (la dé-répression) de *VRN1*, et donc la floraison dans de nombreux cultivars de blé et d'orge. La relation entre ces gènes peut également expliquer un fait connu depuis longtemps que les cultivars d'orge facultatifs, qui présentent une délétion dans le locus *VRN2*, commencent à fleurir beaucoup plus tôt en période de jours longs qu'en période de jours courts.

2.4 ÉPIGÉNÉTIQUE

Le génome désigne l'ensemble du matériel génétique d'un individu. Son support, l'ADN, contient toutes les séquences codantes et non codantes transmises d'une génération à l'autre. Pourtant, au regard du caractère unique de chaque individu, la génétique ne parvient pas à expliquer l'ensemble des mécanismes de différenciation observés. Chacune des cellules d'un individu possède le même patrimoine génétique mais elle ne développe pas les mêmes caractéristiques. Depuis quelques années, contrairement à l'idée répandue selon laquelle nous sommes programmés par notre code génétique, des scientifiques ont montré que celui-ci est en réalité une banque de données qui peuvent être activées ou non selon nos conditions de vie (nutritionnelles et psychiques). Il y a eu une accumulation de preuves scientifiques que la qualité de l'environnement et le vécu psychologique modifient l'état des gènes par la voie épigénétique, en les activant ou en les réprimant. Des observations immédiates sur les plantes illustrent le fait que les graines issues d'une plante donnée, donc possédant le même code génétique, produisent des plantes d'aspect bien différent selon le terrain où elles sont semées, selon la façon dont on les a nourries. De même, tous les êtres vivants dépendent de leur nourriture et de leur environnement pour se développer et s'épanouir. La croissance d'un organisme dépend donc à la fois du code génétique et de sa mise en œuvre par des facteurs

environnementaux adéquats (eau, nourriture, soins, etc.). Dans sa définition, le terme épigénétique désigne des paramètres, transmissibles au cours des divisions cellulaires et qui contribuent à la régulation d'états fonctionnels au sein d'une cellule sans affecter directement la séquence d'ADN. L'épigénétique est le domaine qui étudie comment l'environnement et l'histoire individuelle influent sur l'expression des gènes sans altération de l'ADN.

Alors qu'on imaginait récemment encore que les maladies étaient dues essentiellement à la génétique, il est maintenant clair que de nombreuses pathologies, telles que certaines formes de cancer sont associées à des altérations épigénétiques, et cela change complètement les perspectives de traitement. Le cancer peut avoir une origine génétique lorsqu'un gène est touché par une mutation, une délétion ou une translocation chromosomique. Mais on sait désormais que les anomalies épigénétiques sont au moins aussi importantes dans ce type de pathologie. Les anomalies épigénétiques les mieux connues touchent la méthylation de l'ADN. Une hyperméthylation du gène p53 (anomalie épigénétique) qui a pour rôle biologique la transcription de gènes du cycle cellulaire, de l'apoptose et de la réparation de l'ADN peut conduire aux cancers du poumon, de la prostate, du sein et de l'ovaire (Deltour, Chopin et Leprince, 2005). Ce phénomène, responsable de l'inactivation de ces gènes, rend donc plus facile la transformation tumorale de la cellule affectée. De nouveaux médicaments agissant pour diminuer la méthylation de l'ADN ont été développés et peuvent être désormais associés à certaines thérapies pour combattre le cancer. Il est nettement plus facile de modifier la méthylation de l'ADN que de changer la séquence d'ADN sous-jacente. En principe, tout facteur environnemental, nutritionnel et physique qui conduisent à des mécanismes de régulation transmissibles à un génome sans en altérer la séquence primaire nucléotidique pourraient être considérés comme facteurs épigénétiques.

2.4.1 ORGANISATION DE L'ADN

Le génome nucléaire s'organise en une structure nucléoprotéique, appelée chromatine, qui, outre sa composante génétique est riche d'une information épigénétique (Kornberg, 1977 ; Wolffe, 1998). Cette information épigénétique au sein de la chromatine est principalement véhiculée par des modifications de l'ADN et des histones. De plus, contrairement aux modifications génétiques, les modifications épigénétiques sont dynamiques et réversibles. La modification majeure de l'ADN est la méthylation de la cytosine, qui est généralement la marque d'une chromatine transcriptionnellement silencieuse chez les vertébrés (Bird, 2002). La structure moléculaire est composée de, son unité élémentaire, le nucléosome qui comprend une particule cœur et une région internucléosomique. La particule cœur est composée de 146 paires de bases d'ADN enroulées autour d'un octamère protéique comprenant deux copies de chacune des histones H2A, H2B, H3 et H4 (Luger, 2003), la région internucléosomique étant pour sa part caractérisée par la présence de l'histone H1 (histone « lien »). C'est donc la chromatine, et non l'ADN seul, qui est impliquée dans tous les évènements moléculaires faisant intervenir le matériel génétique, à savoir la réplication, la transcription, la réparation et la recombinaison.

Les histones sont les pièces maîtresses de la compaction de l'ADN en chromatine et jouent un rôle majeur dans la régulation des fonctions du génome. Elles sont les cibles de multiples modifications post-traductionnelles qui apportent une information épigénétique. L'ensemble de ces modifications constituerait un « code histone », permettant d'associer à chaque combinaison de modifications un état particulier de la chromatine. Ce répertoire élargi d'informations permet d'envisager de nouvelles possibilités de régulation épigénétique. L'importance cruciale des modifications épigénétiques au cours du développement normal et leur implication dans de nombreuses maladies ne font plus aucun doute. Outre son intérêt théorique

dans le décryptage des mécanismes fondamentaux de la régulation de l'expression des gènes, l'épigénétique ouvre également des perspectives fascinantes pour l'utilisation de nouvelles approches thérapeutiques. Par ailleurs, il a été démontré que le nombre de CpG méthylés augmentait avec l'âge, contribuant en partie au développement de maladies chroniques. Toutefois, bien que plusieurs agents inhibant la méthylation aient été découverts à ce jour, la plupart ne sont pas spécifiques des méthyltransférases de l'ADN. Les récents progrès concernant la compréhension des phénomènes épigénétiques permettent d'envisager dès à présent des approches plus rationnelles, notamment au niveau de la modélisation moléculaire d'agents ciblant spécifiquement ces modifications épigénétiques. Quant aux histones, différentes modifications post-traductionnelles ont été décrites, comme l'acétylation, la phosphorylation, la méthylation et l'ubiquitinylation (Jenuwein et Allis, 2001 ; Turner, 2002). La plupart des enzymes régulant les modifications de la chromatine sont connus et ont été largement étudiées ; elles comprennent des histones acétyltransférases, des désacétylases, méthyltransférases et, plus récemment, des histones déméthylases (Shi, 2007).

2.4.2 LA MÉTHYLATION DE L'ADN

La modification covalente de l'ADN par la méthylation de la cytosine est un processus épigénétique héréditaire et réversible qui est impliqué dans la régulation d'un large éventail de processus biologiques chez les animaux vertébrés, les plantes et les champignons (Colot et Rossignol, 1999). L'ajout d'un groupement méthyle à la base cytosine précédent la base guanine (le dimer CpG) ne change pas la séquence primaire de l'ADN et est donc considéré comme une modification épigénétique. Il y a environ 40 ans depuis que la méthylation de l'ADN a été postulée comme étant une modification héréditaire capable d'influencer l'expression du gène (Holliday et Pugh, 1975 ; Riggs, 1975). Trois enzymes à activité catalytique, l'ADN méthyltransférase 1

(DNMT1), DNMT 3a et DNMT 3b sont nécessaires pour l'établissement et le maintien du profil de méthylation de l'ADN (Bird, 2002). Deux enzymes supplémentaires, DNMT 2 et DNMT 3L, présentent une forte homologie et sont exprimés chez plusieurs types cellulaires, y compris les cellules souches embryonnaires (Bestor, 2000). La méthylation de l'ADN est généralement considérée comme une modification répressive et est associée à la répression de l'expression génique (Bird, 2002). Cette modification est essentielle pour le développement normal. Chez la souris, la perte de la maintenance de l'enzyme méthyltransférase DNMT1 conduit à une létalité embryonnaire induite par plusieurs déficiences des principaux organes de l'embryon (Li, Bestor et Jaenisch, 1992). En revanche, les souris déficientes en DNMT 3a peuvent se développer jusqu'à terme, mais deviendront chétives et mourront un mois environ après leur naissance (Okano *et al.*, 1999).

2.4.3 L'ACÉTYLATION DES HISTONES

Ce n'est que trente ans après leur découverte, en 1996, qu'on a su que les histones pouvaient être acétylées, avec la première histone acétyltransférase (HAT) identifiée comme ayant un rôle de coactivateur transcriptionnel. Depuis, de nombreuses enzymes ont été identifiées comme ayant une activité HAT. On les classe en deux familles : les MYST (Moz, Ybf2/Sas3, Sas2, Tip60) et les GNAT (Gcn5-related N-Acetyltransferase) (Roth, Denu et Allis, 2001).

2.4.4 LA MÉTHYLATION DES HISTONES

La méthylation peut survenir sur les lysines ou sur les arginines permettant de classer les histones méthyltransférases (HMTs) selon l'acide aminé modifié. Les histones méthyltransférases (HMTs) sont des enzymes (histone-lysine N-

méthyltransférase et histone-arginine N-méthyltransférase) qui catalysent le transfert d'un à trois groupes méthyles à partir du cofacteur S-adénosyl méthionine à des résidus lysine et arginine des protéines histones. Ces protéines contiennent souvent un domaine catalytique SET (Su (var) 3-9, Enhancer of Zeste, Trithorax (Roh *et al.*, 2006)). Les PRMT (Protein Arginine Methyltransferases) peuvent méthyler les arginines tandis que les protéines à domaine SET (Su(var)3-9, Enhancer of Zeste, Trithorax), peuvent méthyler les lysines (Zhang et Reinberg, 2001). Les lysines et les arginines ciblées sont en majorité situées sur les extrémités N-terminales des histones H3 et H4. Chez les eucaryotes supérieurs, la méthylation de la lysine 9 de H3 est retrouvée dans l'hétérochromatine alors que la méthylation de la lysine 4 de H3 est liée à l'euchromatine (Jenuwein et Allis, 2001). Il a aussi été montré que le nombre de groupements méthyles présents sur une même lysine peut mener à différents types de réponses, augmentant ainsi la complexité du code des histones (Santos-Rosa *et al.*, 2002). Un important débat existe autour de la méthylation, à savoir si elle est une modification permanente ou réversible, i.e. s'il existe des déméthylases d'histones. Cette question reste toujours sans réponse mais plusieurs hypothèses ont été émises incluant la protéolyse des domaines N-terminaux des histones méthylées (Bannister, Schneider et Kouzarides, 2002). Deux familles importantes et bien conservées de protéines, le Trithorax et Polycomb, ont des domaines catalytiques SET contenant des histones méthyltransférases spécifiques pour H3K4me3 et H3K27me3, respectivement. Le complexe polycomb répressive 2 (polycomb repressive complex 2 : PRC2) se compose de trois sous-unités, les Suz12, Ezh2 et Eed. La fonction d'EeD et Suz12 restent inconnus, tandis qu'Ezh2, est l'histone-méthyltransférase qui contient le domaine catalytique SET (Bernstein, Meissner et Lander, 2007). Bien que H3K4me3 et H3K27me3 sont généralement enrichis au niveau des régions de chromatine actives et inactives, respectivement, des études ont montré qu'ils co-localisent dans certaines régions génomiques (Bernstein *et al.*, 2006 ; Roh *et al.*, 2006). Il a été suggéré que ces modifications bivalentes régulent l'expression des

gènes du développement soit en les activant ou en les réprimant lors de la différenciation des cellules souches embryonnaires (Bernstein *et al.*, 2006).

2.4.5 LA PHOSPHORYLATION

La majorité des travaux effectués sur cette modification a été réalisée sur la sérine 10 de l'histone H3. Chez la levure *S. cerevisiae*, cette phosphorylation survient lors de l'activation transcriptionnelle et lors de la condensation des chromosomes mitotiques, la modification pouvant être réalisée respectivement par les kinases Snf1 et Ipl1/Aurora (Berger, 2002). La phosphorylation des histones joue aussi un rôle dans la réparation de l'ADN ayant subi des cassures doubles brins. Chez la levure, Mec1 phosphoryle la sérine 129 de l'histone H2A en réponse à certains dommages à l'ADN alors que le résidu 139 de l'histone variante H2A.X est ciblé par une kinase ATM/ATR dans des conditions similaires chez les mammifères (Downs, Lowndes et Jackson, 2000 ; Redon *et al.*, 2002).

2.4.6 L'UBIQUITINATION

L'ajout de plusieurs copies d'ubiquitine est très souvent lié à la dégradation par le protéasome de la protéine modifiée. Cette modification sur les histones est particulière car elle implique une simple mono-ubiquitination non dégradative d'une lysine dans le domaine carboxy-terminal des histones H2A ou H2B. Cette modification a été proposée comme ayant un rôle dans la spermatogenèse, la réponse au stress, ainsi que dans la formation de l'hétérochromatine et dans la régulation de la transcription (Moore, Jason et Ausio, 2002). Rad6 est l'enzyme responsable de l'ubiquitination de l'histone H2B chez la levure (Briggs *et al.*, 2002).

2.4.7 RÉGULATION ÉPIGÉNÉTIQUE DE L'EXPRESSION DU MICROARN

Parmi les nombreux contrôles biochimiques des effets épigénétiques dans l'expression des gènes, la méthylation d'ADN peut être le plus dominant (Guil et Esteller, 2009). La plupart des modifications d'ADN impliquent une modification covalente par méthylation sur les bases cytosine des dinucléotides CpG, la structure principale des îlots CpG. L'hyperméthylation des îlots CpG dans les promoteurs des microARN (miARN) supprime leur transcription (Liang, Bates et Wang, 2009). En outre, la méthylation de l'ADN dans les cellules de mammifères est largement dictée par trois composants protéiques majeurs, DNMT1, DNMT3A et DNMT3B, de la famille de l'ADN méthyltransférase (DNMTs) (Guil et Esteller, 2009). Évidemment, la méthylation des promoteurs de miARN peut être cruciale pour déterminer de la capacité de transcription des miARNs avec succès, et la dé-régulation biochimique de cet événement peut provoquer la perte des miARNs essentiels, et l'évolution ultérieure de pathologies dans les tissus (Liang, Bates et Wang, 2009). De toutes les méthylations de l'ADN, la modification des histones, et la réparation de l'ADN sont des processus biochimiques essentiels, nécessaires au maintien du mode d'expression adéquate des miARNs, les facteurs impliqués dans ces trois processus, contrôlant ainsi l'expression des miARNs ont évolué comme «régulateurs épigénomiques du processus de vieillissement » (Liang, Bates et Wang, 2009).

2.5 MicroARNs

Les miARN sont des ARN non-codants (20-24 nucléotides de long) qui régulent négativement l'expression de leurs gènes cibles par l'intermédiaire soit des séquences spécifiques de dégradation ou de la répression de la traduction de l'ARN en protéines (Carrington et Ambros, 2003). L'explosion récente de la recherche sur le

rôle des petits ARN non codants (microARNs) dans le contrôle de l'expression des gènes a révélé des implications multiformes de ces microARNs en allant de la détermination du destin cellulaire au cours du développement à la maintenance des états définitifs de différentiation cellulaire. Les microARN sont impliqués dans les voies de signalisation régulant le contrôle des processus normaux d'apoptose, de cycle cellulaire, de la différenciation et de l'organisation du cytosquelette, etc ; au niveau post-transcriptionnel, ils sont impliqués soit par la dégradation des transcrits de leurs gènes cibles ou par l'inhibition de la traduction au niveau de la région 3 'non codante. Il n'est pas surprenant que la dérégulation de l'expression de microARN a été liée à la pathogenèse de diverses maladies, parmi lesquelles le cancer, les troubles cardio-vasculaires, et la neurodégénérescence (Wang, 2009).

Chez les plantes, une décision importante est prise quant à savoir si une graine doit germer ou non au cours des étapes précoces de leur développement. Le passage de la graine de la phase maturation à la phase de germination est un changement fondamental dans le programme de développement des semences (Preston *et al.*, 2009). Les microARNs joueraient un rôle crucial dans l'élimination de tout facteur indésirable durant cette transition pour maintenir le développement normal de la plantule. La régulation de facteurs de transcription ciblés par des microARN est impliquée à ces stades critiques du développement des plantes (Martin *et al.*, 2010). Divers processus de développement des plantes, tels que la germination des graines, le développement des semis et la transition de la phase végétative à la transition de phase de reproductive sont régulées par les microARNs (Preston *et al.*, 2009). Les microARNs et leurs gènes cibles sont également associés à l'activation du méristème apical de la tige (SAM), au développement de la feuille primordiale, des feuilles ou des cotylédons embryonnaires et dans la détermination du taux de l'émergence des feuilles végétatives (Nonogaki, 2010).

2.5.1 LES MicroARNs, MOLÉCULES RÉGULATRICES IMPLIQUÉES DURANT LE CYCLE DE VIE DE LA PLANTE

Chez les plantes, les microARN (miARN) sont une classe de molécules régulatrices qui jouent un rôle important tout au long de leur cycle de vie (Lee *et al.*, 2010). Le rôle important des miARN dans la croissance des plantes et leur développement a été démontré par des criblages génétiques des miARN et / ou leurs cibles et des prédictions bioinformatiques basée sur la complémentarité de séquence entre miARN et leurs gènes cibles ont été employés (Rhoades *et al.*, 2002). Ces efforts ont révélé l'existence d'un certain nombre de miARN jouant un rôle crucial dans le développement des feuilles (Palatnik *et al.*, 2003), le développement de fleurs (Chen, 2004), le développement des racines (Guo *et al.*, 2005) et la floraison (Aukerman et Sakai, 2003). Grâce à une technique de marquage, il a été démontré que miARN 172 (miR172) cible une sous-famille de facteurs de transcription d'APETALA2 (AP2) et provoque une floraison précoce et perturbe la spécification de l'identité d'organes floraux lorsqu'il est surexprimé chez *Arabidopsis thaliana* (Aukerman et Sakai, 2003). Le microARN miR172 est normalement exprimé d'une manière temporelle, conformément à son rôle proposé du contrôle du temps de floraison. Des analyses de gain de fonction et la perte de fonction indiquent que deux des gènes cibles *AP2-like* agissent normalement comme répresseurs de la floraison, appuyant l'idée que miR172 contrôle le temps de floraison par régulation négative des gènes cibles *AP2-like* (Aukerman et Sakai, 2003). La mise au point des technologies de séquençage ont grandement amélioré l'efficacité de trouver de nouveaux miARN qui fonctionnent dans des conditions diverses et sur différentes espèces végétales (Fahlgren *et al.*, 2007). Ces approches ont révélé que certaines familles de miARN sont bien conservées entre espèces végétales (Barakat *et al.*, 2007), suggérant que le rôle de régulation de nombreux miARN peut être universel chez les plantes.

L'ensemble de tous ces domaines de recherche décrits dans cette revue de littérature sont largement menés chez la plante modèle *Arabidopsis thaliana.* Par contre chez les *triticeae* et en particulier chez le blé, beaucoup reste à faire dans la recherche pour couvrir ces domaines. Ceci est principalement dû au fait que le génotype qui détermine la réponse à la vernalisation et à la photopériode de cette céréale possède à un caractère récessif (*vrn-A1*). De plus, sa complexité génétique, la difficulté d'obtenir des mutants et le fait que le génome du blé ne soit pas entièrement séquencé et disponible rendent plus ardues les études sur cette céréale. Les bases moléculaires de la vernalisation et la floraison chez le blé sont peu connues. Afin d'améliorer l'avancement des études sur la floraison et la vernalisation chez le blé nous émettons cette hypothèse et les objectifs ci-dessous.

2.6 HYPOTHÈSE

L'hypothèse globale de cette thèse doctorale était que les quatre gènes *TaVRN1*, *TaFT1*, *TaVRN2* et *TaVRT-2* sont régulés de façon épigénétique durant la transition florale en réponse de la vernalisation chez le blé. Cette hypothèse fut émise suite à l'analyse des profils d'expression de ces gènes qui montre que chez le blé de printemps le niveau d'expression des activateurs *TaVRN1* et *TaFT1* est élevé voire constitutif pour *TaVRN1* alors que celui des répresseurs *TaVRN2* et *TaVRT-2* est faible. Par contre chez le blé d'hiver nécessitant la vernalisation; les niveaux d'expression des répresseurs *TaVRN2* et *TaVRT-2* sont élevés et leur répression coïncide avec la transition florale donc l'augmentation de l'expression de l'activateur *TaVRN1* (Kane *et al.*, 2005 ; Yan *et al.*, 2004b). Une fois le cycle de développement de la plante terminé par la formation et la maturation des graines, un autre cycle reprend ce qui laisse suggérer la possibilité d'une régulation épigénétique de ces quatre gènes.

De façon specifique, nous avons voulu savoir :

Quel serait l'impact de la surexpression de *TaVRN2* sur la floraison et la tolérance au gel chez la plante modèle *Arabidopsis thaliana*?

Est-ce que le profil d'expression des quatre gènes (*TaVRN1, TaFT1*, *TaVRN2* et *TaVRT-2*) suggère qu'ils sont régulés de façon épigénétique en réponse à la vernalisation ?

Est-ce qu'une délétion du gène *VRN1* pourrait renseigner de la fonction biochimique de VRN1 chez le blé ?

2.6.1 OBJECTIFS

Pour vérifier ces hypothèses, nous nous sommes fixés les objectifs suivants.

2.6.2 OBJECTIF GÉNÉRAL

Comprendre les bases moléculaires du processus de vernalisation et de la transition florale chez le blé et leur relation avec la tolérance au gel.

2.6.3 OBJECTIFS SPÉCIFIQUES

Objectif 1 : Étudier la régulation épigénétique des gènes *TaVRN1*, *TaVRN2, TaFT1* et *TaVRT-2* en réponse à la vernalisation chez le blé

Objectif 2 : Étudier les fonctions de *TaVRN2* chez la plante modèle (*Arabidopsis thaliana*) d'autant plus qu'il n'y a pas d'homologue connu chez *Arabidopsis*

Objectif 3: Étudier l'impact de la mutation du gène *VRN-1A* chez *Triticum monoccocum* L. sur la floraison et la tolérance au gel

Objectif 4 : Déterminer la fonction biochimique du gène *VRN1*

Les résultats obtenus à partir de ces différentes études ci-haut menées, ont montré que les gènes clés de la floraison sont modulés de façon épigénétique en réponse à la vernalisation et que cette modulation est associée au niveau de méthylation de la chromatine des gènes *TaVRN1*, *TaFT1* et *TaVRN2* chez le blé. De plus, le gène *VRN2* même s'il n'a pas d'homologue chez *Arabidopsis thaliana* peut induire le retard de floraison chez cette plante. La troisième étude nous a permis de mieux comprendre le rôle essentiel du gène *VRN1* durant la transition de la phase végétative à la phase reproductive et de montrer que le méthyl jasmonate induit le retard de la floraison en réprimant l'expression de *FT1*. Malheureusement les résultats obtenus concernant le gène *TaVRT-2* ne furent pas concluants et cette étude a été suspendue.

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CHAPITRE III

HETEROLOGOUS EXPRESSION OF WHEAT *VERNALIZATION 2* (*TAVRN2*) GENE IN ARABIDOPSIS DELAYS FLOWERING AND ENHANCES FREEZING TOLERANCE

Heterologous expression of Wheat *VERNALIZATION 2* (*TAVRN2*) gene in Arabidopsis delays flowering and enhances freezing tolerance

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3.1 RÉSUMÉ

Le gène vernalisation 2 (*VRN2*), est un répresseur majeur de floraison chez les céréales tempérées qui est régulé par les basses températures et la photopériode. Ici, nous montrons que le gène chez *Triticum aestivum* (*TaVRN2*) est aussi régulé par le stress salin, le choc thermique, la déshydratation, les blessures et l'acide abscissique. Une analyse du promoteur de *TaVRN2* indique que sa région régulatrice possède tous les éléments spécifiques associés à ces contraintes. Cela suggère des effets pléiotropiques de TaVRN2 dans le développement du blé et de son adaptabilité à l'environnement. Pour tester si TaVRN2 peut agir comme un répresseur de la floraison chez des espèces différentes des céréales tempérées, le gène a été exprimé de façon constitutive chez la plante modèle *Arabidopsis*. Les plantes transgéniques n'ont montré aucune altération de la morphologie, mais leur date de floraison a été considérablement retardée par rapport aux plantes contrôles, indiquant que TaVRN2, bien que n'ayant pas d'orthologue chez les *Brassicaceae*, peut agir comme un répresseur de la floraison chez ces espèces. Pour identifier les mécanismes possibles par lesquels le gène *TaVRN2* retarde la floraison chez *Arabidopsis*, le niveau d'expression de plusieurs gènes impliqués dans la régulation de la transition florale a été déterminé. L'analyse indique que le retard de floraison chez les plantes transgéniques (*35S::TaVRN2*) a été associée à un schéma complexe de l'expression des gènes majeurs qui contrôlent la floraison, *FCA*, *FLC*, *FT*, *FVE* et *SOC1*. Ceci suggère que l'expression hétérologue de *TaVRN2* chez *Arabidopsis* peut retarder la transition florale en modulant plusieurs voies inductives de la floraison. En outre, les plantes transgéniques ont montré une tolérance plus élevée au gel probablement due à l'accumulation de *CBF2*, *CBF3* et les gènes *COR*s. Dans l'ensemble, nos données suggèrent que le gène *TaVRN2* pourrait moduler un régulateur commun des deux voies de régulation qui régissent le temps de floraison et l'induction de la tolérance au gel. Les résultats démontrent également que TaVRN2 pourrait être utilisée pour manipuler le temps de floraison et améliorer la tolérance au froid chez d'autres espèces.

Mots clé: ***Arabidopsis,*** **Floraison, Tolérance au gel, Expression de gènes, Vernalisation, Blé**

3.2 ABSTRACT

The vernalization gene 2 (*VRN2*), is a major flowering repressor in temperate cereals that is regulated by low temperature and photoperiod. Here we show that the gene from *Triticum aestivum* (*TaVRN2*) is also regulated by salt, heat shock, dehydration, wounding and abscissic acid. Promoter analysis indicates that *TaVRN2* regulatory region possesses all the specific responsive elements to these stresses. This suggests pleiotropic effects of TaVRN2 in wheat development and adaptability to the environment. To test if TaVRN2 can act as a flowering repressor in species different from the temperate cereals, the gene was ectopically expressed in the model plant *Arabidopsis*. Transgenic plants showed no alteration in morphology, but their flowering time was significantly delayed compared to controls plants, indicating that *TaVRN2*, although having no ortholog in *Brassicaceae*, can act as a flowering repressor in these species. To identify the possible mechanism by which *TaVRN2* gene delays flowering in *Arabidopsis*, the expression level of several genes involved in flowering time regulation was determined. The analysis indicates that the late flowering of the 35S::*TaVRN2* plants was associated with a complex pattern of expression of the major flowering control genes, *FCA*, *FLC*, *FT*, *FVE* and *SOC1*. This suggests that heterologous expression of *TaVRN2* in *Arabidopsis* can delay flowering by modulating several floral inductive pathways. Furthermore, transgenic plants showed higher freezing tolerance, likely due to the accumulation of *CBF2*, *CBF3* and the *COR* genes. Overall, our data suggests that *TaVRN2* gene could modulate a common regulator of the two interacting pathways that regulate flowering time and the induction of cold tolerance. The results also demonstrate that *TaVRN2* could be used to manipulate flowering time and improve cold tolerance in other species.

Key words: ***Arabidopsis,*** **Flowering, Freezing tolerance, Gene expression, Vernalization, Wheat**

3.3 INTRODUCTION

In temperate regions, low temperature (LT) constitutes a major factor that regulates flowering time and many developmental transitions such as germination, bud dormancy and bursting (Henderson, Shindo et Dean, 2003). In response to LT-conditions, plants cold acclimate and vernalize to prevent the sensitive floral meristem from freezing damages during the winter by postponing flowering (Fowler *et al.*, 2001). The ability of plants to switch from vegetative to reproductive phase after a long period of cold, a process known as vernalization (Chouard, 1960), allows plants to promote flowering early in the spring. During this cold exposure period, LT-responsive genes *CBFs* (*C-repeat binding factor*) and *COR* (*Cold Regulated*) are activated, allowing plants to increase their tolerance to cold and survive the winter (Thomashow, 1990). Cereals are classified into spring and winter growth habit according to their vernalization requirement (Michaels *et al.*, 2003 ; Pugsley, 1972). Spring varieties do not respond to vernalization and flower rapidly whereas winter varieties have a quantitative vernalization requirement. Therefore, winter varieties require vernalization to accelerate flowering and complete their life cycle. Understanding the genetic/molecular basis of both LT-responsive pathways (cold acclimation and vernalization), can help to better manipulate, the two important agronomical traits flowering and freezing tolerance.

In *Arabidopsis thaliana*, two key loci *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) determine the difference in the flowering time and freezing tolerance (Koornneef *et al.*, 1994 ; Napp-Zinn, 1987). Spring ecotypes such as *Landsberg erecta* and *Columbia* have a non-functional *FRI* and/or a weak allele of *FLC* whereas winter annuals such as *Stokholm* and *San Felieu2* have functional *FRI* and *FLC* genes (Johanson *et al.*, 2000 ; Michaels *et al.*, 2003)). *FLC*, a MADS-box gene, is the central regulator of flowering through the vernalization pathway. A high

level of *FLC* expression (RNA and protein) induces a very late-flowering phenotype in winter annual ecotypes. In contrast, repression of *FLC* by vernalization causes an early-flowering phenotype (Michaels et Amasino, 1999 ; Sheldon *et al.*, 1999). A recent study showed that FRI, which encodes a novel nuclear protein with no conserved domains except for two coiled coil regions, delays flowering in *Arabidopsis* through a co-transcriptional mechanism involving direct interaction with the nuclear cap binding complex, with concomitant effects on *FLC* mRNA splicing and transcription (Geraldo *et al.*, 2009).

In cereals, vernalization and cold acclimation regulatory gene networks are also interconnected but are regulated by different factors. It was hypothesized that the temperate cereals have evolved the ability to use the presence of *VRN1* in the leaves as a signal to down regulate the *COR* genes (Danyluk *et al.*, 2003). This hypothesis does not necessarily imply a direct interaction between *VRN1* and *CBF* or *COR* genes (Galiba *et al.*, 2009). However, the molecular and genetic mechanisms involved in the association between the up regulation of *VRN1* and the down regulation of *COR* genes are not yet determined. Genetic analysis and expression profiling studies in wheat showed that *VRN1* transcript accumulation is associated with the vernalization response and the transition from the vegetative to the reproductive phase (Danyluk *et al.*, 2003 ; Murai *et al.*, 2003 ; Yan *et al.*, 2003). *VRN1* encodes a FRUITFULL-like MADS-box protein that belongs to the AP1/SQUA-like clade of transcriptional regulators whose members have been implicated in meristem identity and flower development (Ferrandiz, Liljegren et Yanofsky, 2000 ; Mandel et Yanofsky, 1995). VRN2 is a zinc finger transcription factor that acts as a dominant negative regulator of flowering time in the vernalization pathway in wheat and barley (Karsai *et al.*, 2005 ; Szucs *et al.*, 2007 ; Yan *et al.*, 2004). Reducing *VRN2* level by RNAi accelerates flowering in hexaploid wheat (Yan *et al.*, 2004). Thus, there are obvious differences in the flowering regulation pathway between *Arabidopsis* and cereals. No genes corresponding to *FLC* were found in monocots (Hecht *et al.*, 2005 ; Lee *et al.*,

2005) as well no TaVRN2 orthologs were detected in *Brassicaceae* (Yan *et al.*, 2004). These observations suggest that monocots and dicots have separately evolved their vernalization pathway recruiting different components for analogous functions. However, the reciprocal control of flowering by the rice *SOC1* gene in transgenic *Arabidopsis* and by the *Arabidopsis FLC* gene in transgenic rice (Tadege *et al.*, 2003) was an indication that the flowering genes from one species could be used to manipulate flowering time in other species. In support of this hypothesis we showed that overexpressing of wheat MADS-box genes *TaVRN1* or *TaVRT2* modulates flowering time in *Arabidopsis* (Adam *et al.*, 2007). This cross species function of flowering genes may help to understand the evolution of the molecular mechanisms underlying vernalization and flowering time in plants.

In the present study, we provide further evidence of the cross species function of flowering genes. We showed that the wheat vernalization gene *TaVRN2* can act as a flowering repressor outside the cereal group. *Arabidopsis* transgenic plants expressing *TaVRN2* showed a late-flowering phenotype and enhanced freezing tolerance without any alteration in their morphology. These modifications are associated with changes in the expression of the flowering and freezing tolerance responsive genes.

3.4 MATERIALS AND METHODS

3.4.1 PLANT MATERIAL AND GROWTH CONDITIONS

Two wheat varieties (*Triticum aestivum*, 2n x 6 = 42), a spring habit cultivar Manitou and a winter habit cultivar Norstar, were grown in a controlled growth chamber as previously described (Danyluk *et al.*, 2003). Briefly, plants were grown in a growth chamber at 20°C under long days (LD) (16 h at 320 μmol m^{-2} s^{-1}) or short days (SD) (8 h at 320μmol m^{-2} s^{-1}) conditions. For vernalization treatment, 14-day old plants were grown at 4°C for 0, 7, 14, 21, 28, 35, 42, 49, 56, 77 and 98 days under either SD and LD.

For abiotic stress treatment, 7 day old wheat plants were treated as follows: for cold, seedlings were cold treated for one day at 4°C (CA); for heat shock, they were exposed at 40°C for 1 and 3 h; wounding stress was induced by cutting seedlings into 1 cm segments and placing them in water at 20°C for 3 and 14 h; salt-stressed plants were obtained by incubating seedlings for 18 h with 300 or 500 mM NaCl; water stress was induced by removing seedlings from vermiculite and leaving them at 20°C without water for different time periods, after which the relative water content (RWC) was evaluated; for ABA treatment, 100 mM ABA in 0.02% (v/v) Tween-20 for 18 h were sprayed on seedlings.

For genes expression, flowering time (leaf number) and freezing experiments in *Arabidopsis thaliana*, seeds were put on soil and kept in a cold room (4°C) in the dark for 2 days. After this treatment, seeds were transferred to a growth chamber at 20°C under long days (16 h at 120μmol m^{-2} s^{-1}) conditions. For the cold treatment, 15 day old plants grown at 20°C under long days were transferred to 4°C for four weeks. Flowering time was measured as the number of rosette leaves every week.

3.4.2 CLONING OF *TAVRN2* GENES AND ITS PROMOTERS (SUPPLEMENTARY MATERIAL S2)

One homologous copy of the *VRN2* gene in hexaploid wheat (*TaVRN-B2*) was isolated from wheat cDNAs libraries as previously described (Kane *et al.*, 2007). The second copy, *TaVRN-A2*, was PCR-amplified from genomic DNA (*Triticum aestivum* L.) using specific primers (**Table S1**). Promoters of *VRN2* genes from spring and winter wheat were also PCR-amplified from genomic DNA using specific primers **(Table S1& Sequences S1)**.

3.4.3 CHROMOSOME LOCALIZATION OF *TaVRN-2* GENES

Genomic DNA was extracted from several stocks of the wheat cultivar Chinese Spring: ditelocentric series provided by the USDA from E. R. Sears collection.

From the diluted genomic stocks, 2 µL (20 ng) was used as a template in a 25 µL. PCR reaction containing 1 X TaqMan universal PCR master mix (Invitrogen), 0.9 µM non-fluorescent primers, and 0.25 µM TaqMan-MGB fluorescent probe. The PCR thermal cycling parameters were 50°C for 2 min, 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. At the end of the run, the Ct values were compared, and genetic stocks that showed a delayed or undetectable amplification were identified as the location of the assayed *TaVRN-2* gene.

3.4.4 OVEREXPRESSION OF *TaVRN2* IN *ARABIDOPSIS*

Agrobacterium tumefaciens carrying the binary vector *pGreenII0029/35S::TaVRN-B2* was used to transform *Arabidopsis (Col 0)* using the floral dip method (Clough et Bent, 1998). Wild type and plants transformed with *pBIN19/35S::GUS* were used as controls. T_1 seeds were selected on 0.5x MS salts and

1x MS vitamins containing 50 μg ml^{-1} kanamycin. Transgenic T_1 seedlings were transferred to soil and kept at 20 °C day / night in a growth chamber and grown to maturity under LD conditions (16h photoperiod) at 20°C (day/night) to produce T2 and T3 lines as previously described (Adam *et al.*, 2007). Three independent homozygous lines of the fourth generation were used for the experiments.

3.4.5 DETERMINATION OF FREEZING TOLERANCE

A Caltec Scientific Ltd. Model 8-792 Large Capacity Temperature Stress Chamber was used to perform the FT tests. This instrument consists of four major component systems: a Sanyo Model MDF-792 24.75 ft^3 capacity ultra-low temperature chest freezer, a custom designed stainless steel plenum box with its integral blower and heater (provides air circulation and heating) and an Omega Engineering Inc. Model CN3002 programmable profile controller (monitors the test-chamber air temperature). The controlled action of the heater combines with the constant cooling of the freezer to achieve the desired temperature at any given time.

Non-acclimated (NA) and cold-acclimated (CA) plants were grown in soil for 3 weeks and subjected to the following freezing treatment. The temperature was lowered gradually to –6.5°C for NA and to –10.5°C for CA plants (2°C h^{-1}) and maintained at this temperature for 6 h. The temperature was then gradually increased to 4°C. To determine temperature variability in the freezer, temperatures were monitored by four independent thermocouples T probes distributed in the freezer and connected to an Agilent 3497-0A data acquisition/switch unit. Freezing regimes that showed more than 0.5°C discrepancies between the different probes were rejected. To minimize light stress effect after the freezing treatment, plants were thawed at 4°C for 24 h in the dark and then moved to growth chamber (20°C) under low light for an

additional 24 h before returning to normal light conditions. Representative pictures were taken 2 weeks after the freezing test. Eighteen plants were frozen per line per assay, and the experiment was repeated at least three times.

3.4.6 GENE EXPRESSION STUDIES IN WHEAT

Total RNAs were isolated from wheat plants as described previously (Charron *et al.*, 2005), reverse-transcribed, and subjected to quantitative real-time PCR on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The PCR thermal-cycling parameters were 50°C for 2 min, 95°C for 2 min, followed by 50 cycles of 95°C for 20 sec and 60°C for 1 min. For qRT-PCR, relative transcript abundance was calculated and normalized with respect to 18S ribosomal RNA transcript levels. Data shown represent mean values obtained from four independent amplification reactions, and the error bars indicate the ± SE of the mean. Each experiment was repeated three times. TaqMan primers set were designed according to the specific sequence of the target gene (Invitrogen) (Table S1). All calculations and analyses were performed using SDS RQ Manager 1.1 software using the $2^{-\Delta\Delta}$Ct method with a relative quantification (RQ)min/RQmax confidence set at 95% (Livak et Schmittgen, 2001). The error bars display the calculated maximum (RQmax) and minimum (RQmin) expression levels that represent SE of the mean expression level (RQ value). The upper and lower limits define the region of expression within which the true expression level value is likely to occur (SDS RQ Manager 1.1 software user manual; Applied Biosystems). Amplification efficiency (98% to 100%) for the two primer sets was determined by amplification of cDNA dilution series using 80, 20, 10, 5, 2.5, and 1.25 ng per reaction (data not shown). Specificity of the RT-PCR products was assessed by gel electrophoresis. A single product with the expected length was obtained for each reaction.

3.4.7 GENE EXPRESSION IN *ARABIDOPSIS*

Total RNA was extracted from leaves harvested in the middle of the day and transcript level of flowering genes was measured by qRT-PCR using Syber Green. The PCR thermal-cycling parameters were 94°C for 2 min, followed by 40 cycles of 94°C for 20 sec and 58°C for 1 min and an extension of 72°C for 10 min. Each value is the mean of four separate qPCR reaction normalized to *ACTIN2*. Each experiment was repeated two times using RNA prepared from two biological samples with similar results.

For *AtCBFs* and *AtCORs* genes expression analysis, total RNAs were isolated from the leaves of *Arabidopsis* plants at different stages as described for each experiment. RT-PCR analyses were performed using SuperScript™ First-Strand Synthesis System for RT-PCR KIT according to instructions (Invitrogen).
Specific probes of flowering and cold associated genes used are presented in Table S1. All the experiments were repeated at least three times with three biological replicates.

3.4.8 STATISTICAL ANALYSIS

Results were expressed as mean_SEM of three experimental repeats using different plants. Comparison between groups and analysis for differences between means of control and treated groups were performed using ANOVA followed by the post-hoc test Newman–Keuls ($P<0.05$). The threshold for statistical significance was: *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ and ns: $P>0.05$.

3.5 RESULTS

3.5.1 STRUCTURAL ANALYSIS OF *VRN2* IN *T. aestivum*

Two *TaVRN2* sequences were cloned from *T. aestivum* cv Norstar. The first sequence of 642 bp length was PCR-amplified from wheat cDNA libraries and the second copy of 621bp length was amplified from genomic DNA (Sequences S1). Alignment at the nucleotide level showed 97% identity between the two sequences with deletion of 21 bp in the first exon and several single nucleotide polymorphisms (SNPs). BLAST search at the DNA level revealed that the expressed sequence cloned from the cDNA libraries shared 96% identity with *TmVRN2* (AY485963.1) from the *T. monoccocum* that carries the diploid genome (AA). This relative low identity suggests that the 642 bp sequence is not the copy A. However, it shows a very good score with the Zinc finger-CCT domain gene (*ZCCT1-B1*) of *Triticum turgidum* ssp. dicoccon; AB genome. A homology search also shows that the 621 bp sequence has 99% identity with the A genome of *TdVRN2* (AY485979.1) indicating that the 621 bp could be assigned as the copy A of *TaVRN2*. Using genomic DNA extracted from ditelocentric wheat lines, the 621 bp sequence was mapped on chromosome 5A whereas the 642 bp sequence was mapped on chromosome 4B as recently reported (Distelfeld *et al.*, 2009). Together with the DNA homology, the data indicate that the 642 bp sequence represents the copy B of *TaVRN2* gene (so-called *TaVRN-B2*) and the 621 bp sequence represents the copy A of the gene (*TaVRN-A2*).

At the protein level, TaVRN-A2 has a seven amino acid deletion (His 49-His 55) compared to TaVRN-B2 (Fig. 1B). Structural analysis of these two proteins indicated the presence of a zinc-finger DNA binding domain located in the first exon and a CCT (CONSTANS, CONSTANS-like and TOC) dimerization domain in the second exon (Fig. 1A). The protein alignment of the two copies is shown in figure 1B. The alignment of the CCT domain of TaVRN-A2 and TaVRN-B2 shows four silent

polymorphisms and a substitution of a cytosine by a thymine base (Fig.1C and 1D). This polymorphism changes the amino acid codon at position 35 of the CCT domain from arginine to tryptophan (Fig. 1C).

3.5.2 *TaVRN2* HOMEOLOGOUS GENES SHOW DIFFERENTIAL EXPRESSION PROFILE

To determine *TaVRN2* expression level, total RNAs were extracted from different parts of the winter wheat seedlings (leaves, crowns and roots) and reverse-transcribed for qRT-PCR analyses. The data indicated that *TaVRN2* transcripts accumulated mostly in leaves, lesser in crown and was barely detectable in roots (Fig.2C). The highest level of accumulation is found in 6-day old leaves from non acclimated plants. In LT-acclimated winter wheat, the level of *TaVRN2* transcripts in leaves declined to reach the lowest level after 6 weeks (Fig.2A). *In situ* hybridization experiments showed that *TaVRN2* transcripts are detected in apices cells of winter wheat during the vegetative state (Kane *et al.*, 2007). Together, these data suggest that the LT signal modulating the expression of *TaVRN2* genes could be perceived by either the leaves or the apices.

To test if *TaVRN2* homeologous genes show differential expression patterns, qRT-PCR were performed using specific primers designed to preferentially amplify the A and B copies. The results indicated that the transcripts of the two homologous genes are regulated by LT exposure in the winter genotype (Fig.2). The transcripts level of the two copies remains high during the early stage of LT exposure and starts to decline toward the vernalization saturation point, where the low temperature treatment no longer reduce the final leaf number (FLN). However, *TaVRN-A2* transcript accumulates to a lesser level compared to *TaVRN-B2* (Fig.2A). The down-regulation of *TaVRN2* transcripts by vernalization is associated with the up-regulation of *TaVRT-1* also called *TaVRN-1* as shown by our previous reports (Kane *et al.*,

2007). The results also showed that the two copies of *TaVRN2* transcripts accumulate to a higher level in winter wheat compared to spring wheat during cold acclimation (Fig. 2A). In addition, *TaVRN-B2* transcripts accumulate to higher level during the first three weeks of cold acclimation in winter wheat grown under LD compared to SD. (Fig. 2B). This result is in agreement with previous data in barley (Trevaskis *et al.*, 2006) that showed vernalization has a greater effect on *HvVRN1* than *HvVRN2* while photoperiod has higher effect on *HvVRN2* than *HvVRN1*. It was also shown that in some genotypes of wheat, the vernalization requirement could be replaced by 6 weeks of short day treatment (Dubcovsky *et al.*, 2006).

3.5.3 *TaVRN2* IS REGULATED BY VARIOUS STRESSES

Sequence analysis of *TaVRN2* promoter region revealed the presence of several putative *cis*-elements involved in cold stress, heat shock, light sensitivity, water stress, wounding and abscissic acid responsiveness (Fig. 3A & Sequences S1). These motifs, except ABRE, are conserved *in T. monoccocum* while barley promoter has only the MBS and DRE. To test the functionality of these motifs, the expression of *TaVRN2* genes was monitored in winter wheat leaves exposed to various abiotic stresses (see the experimental procedures). The results revealed that, *TaVRN-B2* transcripts are repressed by abscissic acid, water stress, heat shock, salt, and wounding treatments (Fig. 3B). The biological significance of the stress regulation of *TaVRN2* requires further investigation.

3.5.4 TaVRN2 ACTS AS A FLOWERING REPRESSOR IN *ARABIDOPSIS*

To determine the function of TaVRN2 in modulating flowering time in *Arabidopsis*, transgenic plants expressing *TaVRN-B2* allele under the control of the

CaMV 35S promoter (*35S::TaVRN2*) were compared with wild type *Arabidopsis* (Col-0) and transgenic plants expressing *GUS* under the control of the *CaMV 35S* promoter (*35S::GUS*). Among the *35S::TaVRN2* homozygous lines showing late flowering phenotypes under LD conditions, ten lines showing different levels of late flowering and transgene expression were randomly selected and brought to the next generation. These observations suggest that there is a correlation between phenotype and the transgene expression level. Based on the strength of the phenotype, seven of these lines were brought to the second generation for further analysis. Three independent lines that conserved the late flowering phenotypes were selected and brought to the third generation. Homozygous plants of the fourth generation were analysed (Fig. 4). The flowering time was measured by the number of rosettes of leaves when flowering occurs. The results show that this number was 10 leaves for control plants, and 15 , 13 and 12 leaves for line1, line2 and line3 respectively (Fig. 4C). This flowering time was delayed by 7 days ($P<0.01$) in line 1 and 5 days in line 2 and 3 when grown under long day conditions confirming that the strength of delay of flowering was consistent with the transgene expression level (Fig. 4). Effect of *TaVRN-B2* expression on flowering initiation and development is shown in Fig. 4A. All control plants initiated flower buds and flower formation after 24 and 28 days respectively compared to the three transgenic lines. After 35 days the midflower formation is completed in control plants while the trangenic lines are still at stage of flower formation. This demonstrates clearly, that *TaVRN-B2* overexpression delays flowering time and development in the dicot *Arabidopsis*.

Since *VRN2* encoded a zinc-finger transcription factor (Yan *et al.*, 2004), we assumed that its ectopic expression in *Arabidopsis* could delay flowering time through similar manner to *Arabidopsis* zing finger CO. To test this hypothesis, the effect of *TaVRN2* on genes involved in the flowering pathway was determined. Quantitative RT-PCR analyses of several flowering genes using RNA extracted from *35S::TaVRN-B2* (Line1) and control lines (*Col-0* and *35S::GUS*) grown under LD at

20°C were performed. The analysis (Figure 5A) showed an increase of *AtFLC* by 4 fold in the *35S::TaVRN-B2* transgenic plants compared to the control. This high expression of the central regulator *FLC* in the *35S::TaVRN-B2* plants during the vegetative phase suggests that *TaVRN2* may execute part of its action on *Arabidopsis* flowering time by acting on *AtFLC* transcription. On the other hand, the transcript of the autonomous pathway genes (*AtFCA* and *AtFVE*) and the flowering inducers (*AtFT* and *AtSOC1*) accumulate in transgenic plants, but this is not enough to induce flowering. We hypothesize that the level of accumulation of floral inducer genes is not sufficient to override the 4 fold increase of *FLC* transcript in transgenic plants.

To determine the effect of cold acclimation on FLC stability, two week plants were cold acclimated for four weeks. Quantitative RT-PCR analyses (Fig. 5B) showed that *AtFLC* transcripts level in *35S::TaVRN-B2* plants were higher during the cold acclimation period compared to control plants. More interestingly, we observed that the control plants start bolting when the level of *AtFLC* decreased to the lower level after two weeks of cold treatment (Fig. 5C). The transgenic plants start bolting two weeks later concomitantly with the decline of *AtFLC* level (Figs. 5B and 5C). These results suggest that the high accumulation of *AtFLC* in the*35S::TaVRN-B2* plants are associated with the late flowering phenotypes.

3.5.5 *TaVRN2* ENHANCES FREEZING TOLERANCE AND UP REGULATES *COR* GENES EXPRESSION IN TRANSGENIC *ARABIDOPSIS*

To test if the delay of flowering and the extension of the vernalization phase are associated with enhanced cold tolerance, freezing tests were performed on non-acclimated and acclimated *Arabidopsis* plants as described in the experimental procedures. Non-acclimated transgenic plants showed higher survival rate after

freezing at -6.5°C compared to the control plants (Fig. 6). After cold acclimation the transgenic plants tolerated freezing of -10.5°C compared to cold acclimated controls (Fig. 6). These data indicated that *Arabidopsis* lines over expressing *TaVRN2* are more freezing tolerant than control lines.

RT-PCR analysis was performed to study the expression of LT responsive *Arabidopsis CBFs* and *COR* genes. The results showed that in *TaVRN2* transgenic plants, the expression level of *AtCBFs* and *AtCOR* genes tested are higher than the controls plants (Fig.7 & Fig.S1). These results suggest that over expressing *TaVRN2* in transgenic plants mimic low temperature molecular response.

3.6 DISCUSSION

Molecular characterization of *VRN2* in hexaploid wheat demonstrates sequence variations between the homologous *TaVRN2* genes. *TaVRN-A2* and *TaVRN-B2* exhibit marked sequence variation and higher level of transcript accumulation. This could be explained by the fact that the A, B and D genomes evolved at different rates (Petersen *et al.*, 2006). This variation also supports the idea that various events of sequence changes have occurred in the evolutionary history of wheat homeologous genomes that diverged about 5.0 – 6.9 million years ago (Allaby, Banerjee et Brown, 1999). Indeed, since the polyploidization event of wheat, significant sequence changes have occurred that might cause mutation or lost of *TaVRN2* in modern (spring) wheat. Alignment of the CCT domain of TaVRN-A2 and TaVRN-B2 shows a point mutation at position 35 of the CCT domain, where a tryptophan (W) substitutes for an arginine (R) amino acid. Mutation within this domain results in that *TaVRN-A2* encodes a non-functional protein as shown previously (Yan *et al.*, 2004). This observation may explain why *TaVRN-A2* is expressed at a very low level to the extent that we could not clone it from our cDNA libraries. It is not known, however, if the gene is expressed into non-functional proteins or is not translated at all.

The *TaVRN2* genes are also regulated by photoperiod, dehydration, wounding, heat shock and ABA stresses. This indicates that *TaVRN2*, besides having a major role in regulating vernalization and photoperiod responses, might integrate signals from other environmental stresses to execute its functions during wheat adaptability and development. This is supported by the presence of several putative regulatory *cis*-elements in its promoter region. Down regulation of the flowering repressor TaVRN2 under stress conditions could be used as a strategy to promote flowering and ensure the survival of the species. Flowering time could also be determined by combinatorial responses as in the case of *TaVRN1* and *TaVRT2*, which are regulated by the

combination of both low temperature and day length (Danyluk *et al.*, 2003 ; Kane *et al.*, 2007 ; Kane *et al.*, 2005). However, the biological significance of the regulation of *TaVRN2* by several stresses requires further investigation.

Transgenic *35S::TaVRN2* lines showed a late-flowering phenotype indicating that the repressive effects of TaVRN2 are recognized by certain proteins of *Arabidopsis* flowering pathways. In these lines, the expression level *AtFVE*, *AtFT* and *AtSOC1* was either slightly increased or similar after 7 days of growth under LD conditions. The increase was more evident after 14 days of growth. However, at both time points, the level of *AtFLC* in transgenic plants was 4 times higher than in control plants. This suggests that despite the observed increase of *AtFVE*, *AtFT* and *AtSOC1*, the *AtFLC* level is still high enough to counteract the action of these flowering inducer genes and delays flowering time in plants overexpressing *TaVRN2* (Figure 5A).

A model where AtCO is recruited by another direct DNA-binding and interacts with AtFLC in *35S::AtCO 35S::AtFLC* transgenic plants to repress AtSOC1 factor was proposed (Hepworth *et al.*, 2002). TaVRN2 protein, a zinc finger transcription factor like AtCO, could bind the CCAAT sequence in the *AtSOC1* promoter and regulates its transcription. This hypothesis remains to be confirmed. Taken together, our data indicates that the late flowering of the 35S::*TaVRN2* plants was associated with a complex pattern of expression of the major flowering control *Arabidopsis* genes *FCA*, *FLC*, *FT*, *FVE* and *SOC1*. This suggests that heterologous expression of *TaVRN2* in *Arabidopsis* can also delay flowering by modulating several floral inductive pathways. In cereals, it has been reported that low temperature and daylength flowering-response pathways are integrated to control expression of VRN3 (the AtFT orthologue) and that might occur through regulation of VRN2 (Hemming Megan N, 2008 ; Yan *et al.*, 2006). In contrast, our data did not show a repression of *AtFT* by *TaVRN2* over-expression, suggesting the possibility of TaVRN2 may act

through another pathway in *Arabidopsis*. Overexpressing *TaVRN2* in mutants of these flowering genes will provide the answer to the exact molecular and cellular function of *TaVRN2* gene in *Arabidopsis*.

It is known that the length of the vegetative phase in vernalization sensitive plants is associated with the development of freezing tolerance (Fowler *et al.*, 2001). Plants lose their capacity to cold acclimate once the vernalization saturation point is achieved. Thus extending the vegetative phase in *Arabidopsis* by overexpressing *TaVRN2* should mimic low temperature response. Our data confirms this hypothesis and showed that the delay in the transition to the reproductive phase is associated with enhanced freezing tolerance. This increase in tolerance is partly due to the induction of *AtCBFs* and *AtCORs* genes. *TaVRN2* could have a dual role by regulating flowering time and response to cold as proposed for *AtFVE* (Kim *et al.*, 2004). However, the exact cellular functions of *TaVRN2* in *Arabidopsis* require further investigation.

Flowering time in plants plays a major role in plant adaptation, particularly in temperate region. Premature flowering initiation has a negative effect on productivity. Thus, identifying key genes involved in regulating this important trait will facilitate the development of molecular markers that will be used in breeding programs. It will be of interest to extend the vegetative phase in cold sensitive species to avoid flowering under unfavorable condition. On the other hand, accelerating flowering could be useful to shorter the life cycle for certain species and thus maximize the use of land resources and increase yield.

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Figure 1: *TaVRN2* structure analysis

A) Gene structure showing the two exons and one intron. Exon1 contains the zinc finger domain and exon2 contains the CCT domain.

B) The alignment of TaVRN-A2 and TaVRN-B2 proteins. Regions of putative zinc finger highlighted in red, CCT domains highlighted in blue and difference of nine amino acids highlighted in yellow.

C) The alignment of CCT domain of the two copies of *TaVRN2* showing their different SNPs (in white and red). The sequence highlighted in yellow in the *TaVRN-A2* corresponds to NcoI restriction site.

D) Proteins alignment of CCT domain, Alignments are done by using Clustal W program from the web.

Figure 2: Transcripts level of *TaVRN-B2 and TaVRN-A2* in both spring and winter wheat during cold acclimation

A) *TaVRN-B2 and TaVRN-A2,* in cold acclimated plants. Wheat plants were grown for 14 days at 20°C under a long day (16 h) photoperiod, transferred to 4°C under identical photoperiods, and then sampled at regular intervals.

B) *TaVRN-B2* in cold acclimated plants under short day and long day conditions. Winter wheat plants were grown for 14 days at 20°C under either a long day (16 h) or a short day (8 h) photoperiod, transferred to 4°C under identical photoperiods, and then sampled at regular intervals. qRT-PCR were done using total reverse-transcribed RNA isolated from wheat aerial part. C) *TaVRN-B2* relative transcripts abundance in different tissues. Winter wheat were grown for 7 days at 20°C. Non-acclimated control plants (NA) were maintained at 20°C for 6 days. Cold-acclimated plants (CA) were transferred at 4°C for 36 days. Total RNA was isolated from leaves, crown and roots, reverse-transcribed and subjected to qRT-PCR. Data shown represent mean values obtained from independent amplification reactions (n = 4), and the error bars indicate the range of possible RQ values define by the SE of the delta threshold cycles (Cts). Experiment was repeated three times with similar results.

Figure 3: Promoter analysis and transcripts level of *TaVRN2* in response to various abiotic stresses

A) Putative regulatory *cis*-elements in *TaVRN2* promoter (**MBS:** MYB binding site involved in drought-inductibilty, **ABRE:** *cis*-acting element involved in abscisic acid responsiveness, **HSE:** *cis*-acting element involved in heat stress responsiveness, **ACE:** cis-acting element involved in light responsiveness, **DRE:** cis-acting element involved in cold stress and TATA-box). The plant CARE and PLACE programs were used for the promoter analysis.

B) Transcripts abundance in winter wheat exposed to various stresses. Seven-day old plants were exposed to different stresses: Non-acclimated (NA), cold acclimated (CA), wounding (W), heat shock (HS), salt (NaCl), water stress (RWC), abscisic acid (ABA).

Data shown represent mean values obtained from independent amplification reactions (n = 4), experiment was repeated three times with similar results.

A statistical difference between each sample and the expression observed in non acclimated plants is indicated by an asterisk on top of each histogram columns. The threshold for statistical significance was: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $P>0.05$.

Figure 4: Analysis of transgenic *Arabidopsis* plants overexpressing *TaVRN-B2*

A) Effect of *TaVRN-B2* expression on flower initiation and development during growth under long day conditions at 20°C. The number of plants is expressed as means SEM (n = 99 plants). A statistical difference measurement from three independent experiments. The threshold for statistical significance is indicated by an asterisk on top of each histogram columns; in vertical, comparison between GUS and the three LINES and in horizontal, comparison between Line1 and two others lines; *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ns: $P>0.05$ and sv: same value, statistical analysis is not possible.

B) Phenotypic effect of TaVRN-B2 overexpression in *Arabidopsis*. Control plants *35S::GUS* (GUS) and transgenic plants *35S::TaVRN-B2* (Line1) were grown under long day conditions at 20°C. Pictures were taken at 7, 14, 28, 42 and 50 days (d).

C) Number of rosette leaves at the time of bolting in control plants (*35S::GUS*) and 3 transgenic lines *35S::TaVRN-B2* (L1–L3). Plants were grown under long-day conditions at 20°C and leaves were counted when the first bolt became visible. The number of leaves is expressed as means SEM (n = 18 plants). A statistical difference measurements from three independent experiments (n =18). The threshold for statistical significance is indicated by an asterisk on top of each histogram columns; comparison between GUS and the three LINES; *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ns: $P>0.05$.

D) Transcript level of *TaVRN2* in controls (wild type (WT) and *35S::GUS*) and transgenic lines (L1-L3). Total RNA was extracted from leaves of 15 day-old plants grown under LD conditions at 20°C. *TaVRN-B2* transcript levels were measured by RT–PCR. Each experiment was repeated three times with three biological replicas.

Figure 5: Effect of *TaVRN-B2* overexpression on *Arabidopsis* flowering genes

A) Expression pattern of flowering genes under unvernalized conditions in control (*35S::GUS*) and transgenic *35S::TaVRN-B2* plants. Seven and 14 days (d) old plants were grown at 20°C under LD conditions. Relative transcript abundance is normalized in relation to the level of each gene at 7 days of *35S::GUS* control sample.

B) Expression pattern of *FLC* under cold acclimation conditions in control (*35S::GUS*) and transgenic *35S::TaVRN-B2* plants (Line 1). Fifteen-day old plants were grown at 4°C for 5 weeks. Relative transcript abundance in normalized to the 35S::GUS control sample at zero time.

C) Total number of leaves (n= 32) of cold acclimated control (*35S::GUS*) and transgenic *35S::TaVRN-B2* plants (Line 1) at bolting stage.

Figure 6: *TaVRN-B2* enhances freezing tolerance in *Arabidopsis*

A) Control and transgenic plants after freezing test. Plants were grown for 3 weeks at 20°C (NA) or grown for 3 weeks at 20°C then transferred to 4°C for 7 days (CA7). Controls plants wild type (WT) and *35S::GUS*), transgenic *35S::TaVRN-B2* lines (Line1-Line3). Plants were subjected to freezing test (NA frozen to -6.5°C and CA7 frozen to -10.5°C). Pictures were captured for the same plants before the freezing and after a recovery period of 2 weeks.

B) Survival rate after freezing stress expressed as a percent of surviving plants.

A statistical difference measurements from three independent experiments (n =33). The threshold for statistical significance is indicated by an asterisk on top of each histogram columns; comparison between GUS and the three LINES; *: $P<0.05$, **: $P<0.01$, ns: $P>0.05$.

Figure 7: Effect of *TaVRN-B2* over-expression on the accumulation of cold-regulated transcripts

A) Transcript level of *CBFs* genes. Control (*35S::GUS*) and transgenic 35S::*TaVRN-B2* plants (line 1) grown under long day conditions and exposed to 4°C for 3 and 6 hours.

B) Transcript level of *CORs* genes. Control (*35S::GUS*) and transgenic *35S::TaVRN-B2* plants (line 1) grown under long day conditions at 4°C for 1 and 2 weeks. Transcripts levels were measured by RT-PCR. Each experiment was repeated three times using RNA prepared from three biological samples with similar results.

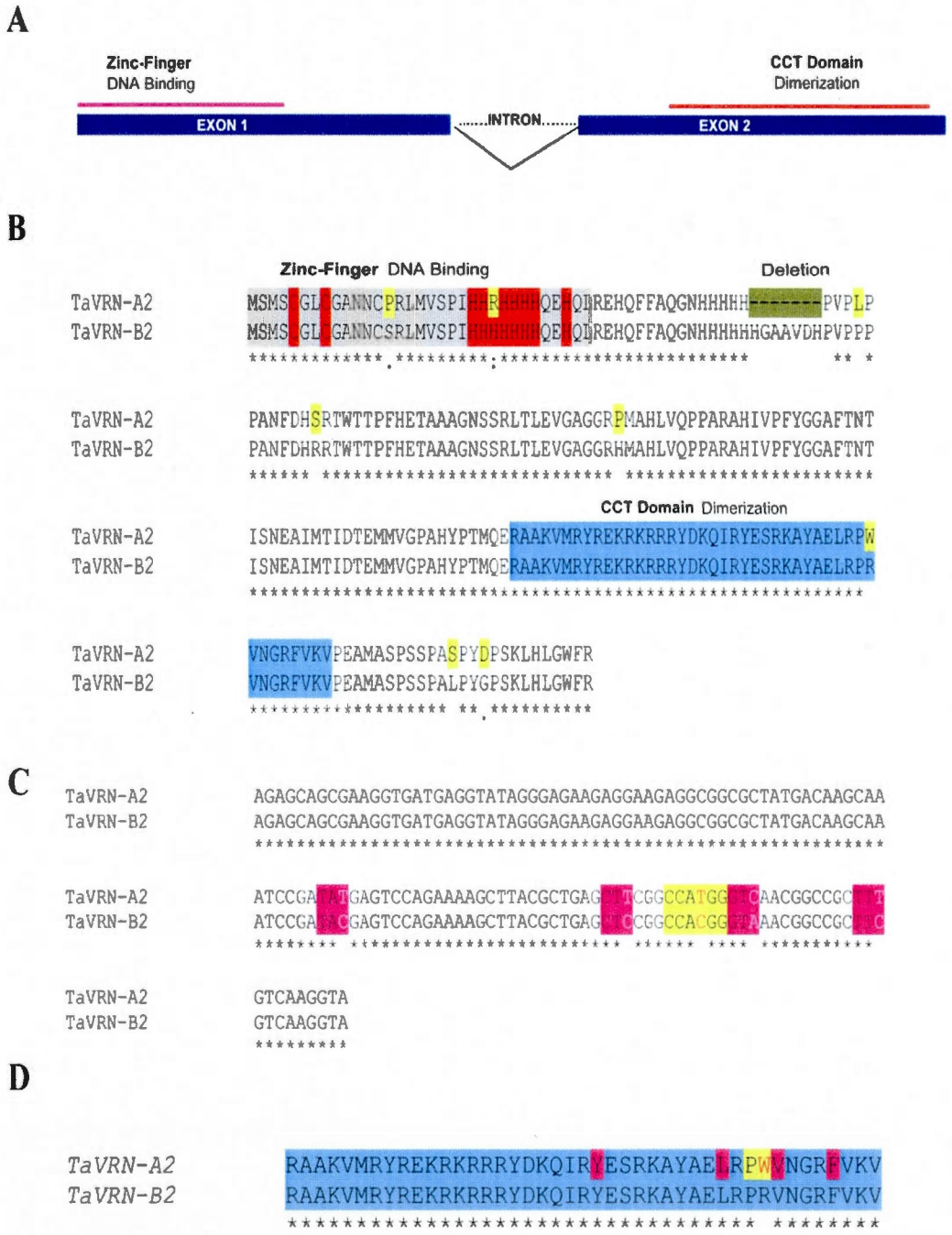


Figure 3.1 *TaVRN2* structure analysis

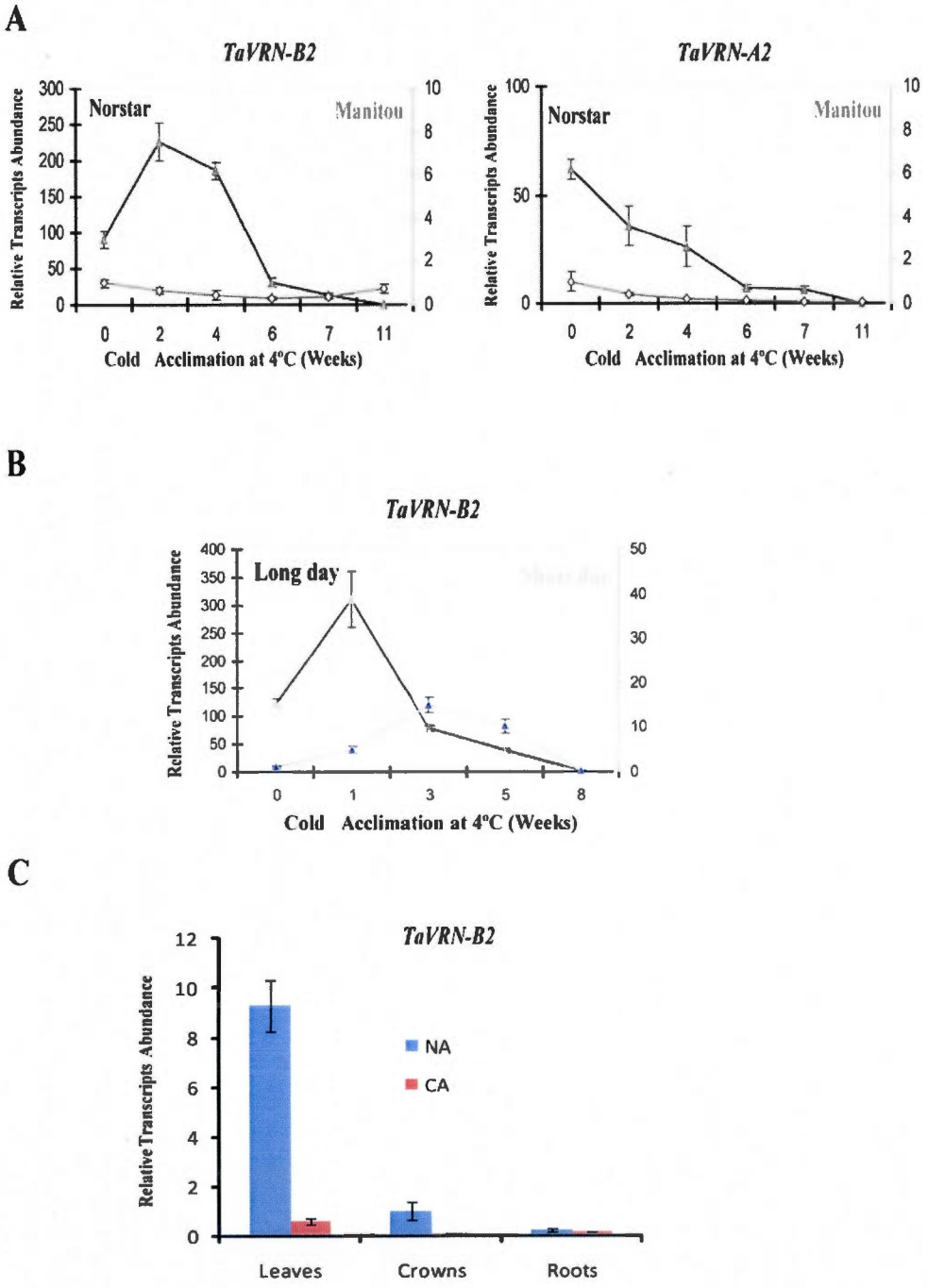


Figure 3.2 Transcripts level of *TaVRN-B2 and TaVRN-A2* in both spring and winter wheat during cold acclimation

A

B

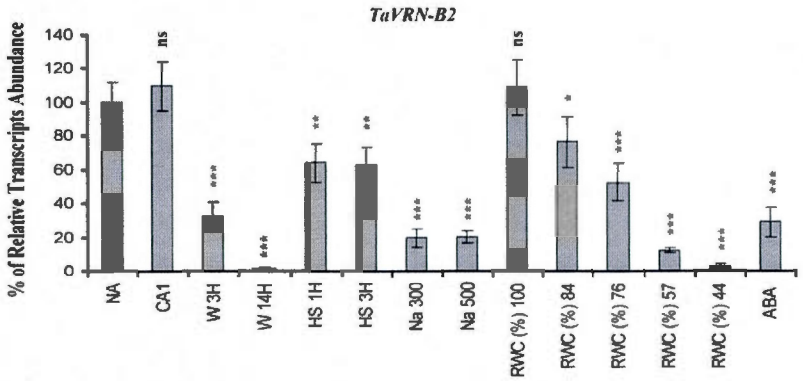


Figure 3.3 Promoter analysis and transcripts level of *TaVRN2* in response to various abiotic stresses

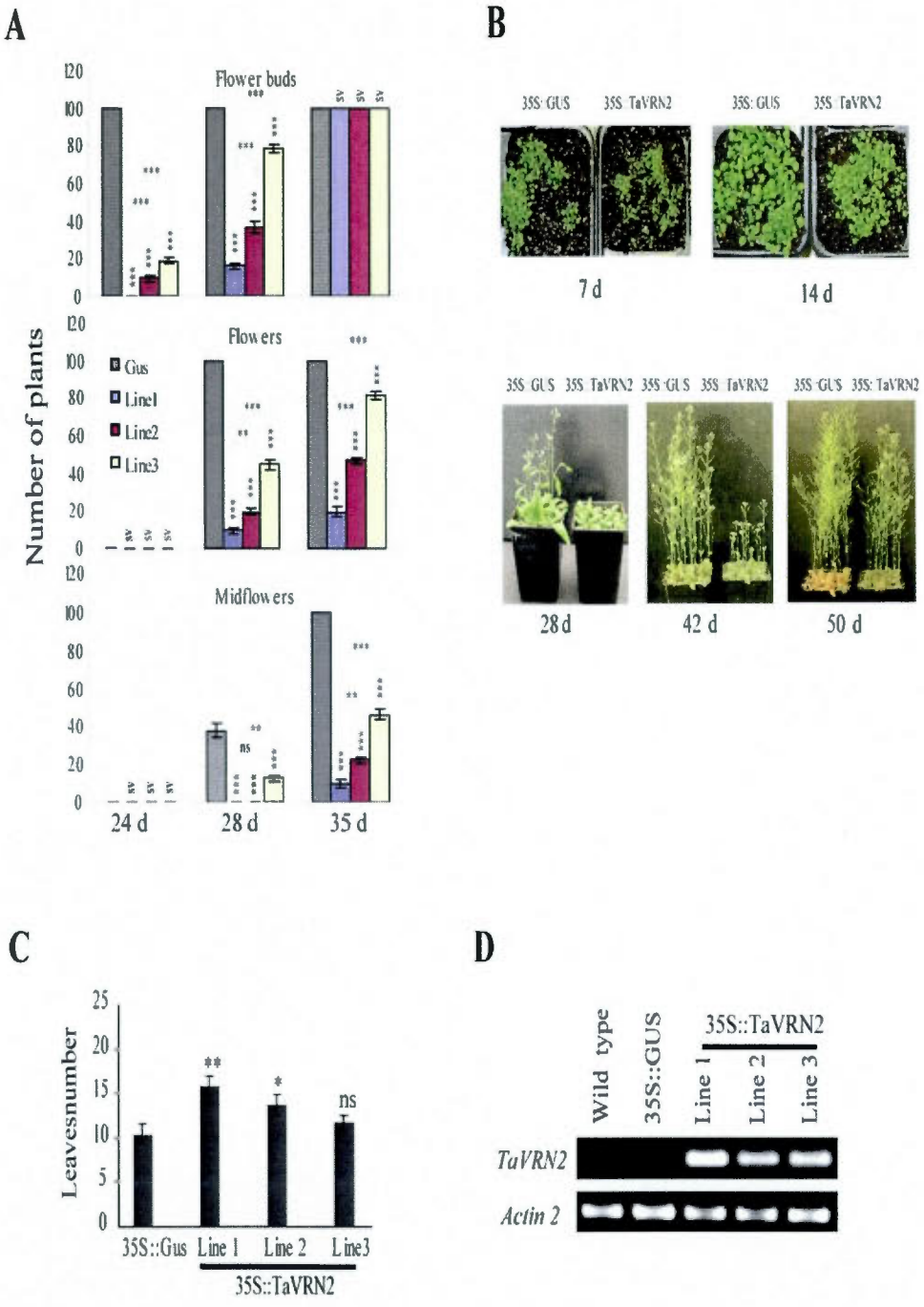
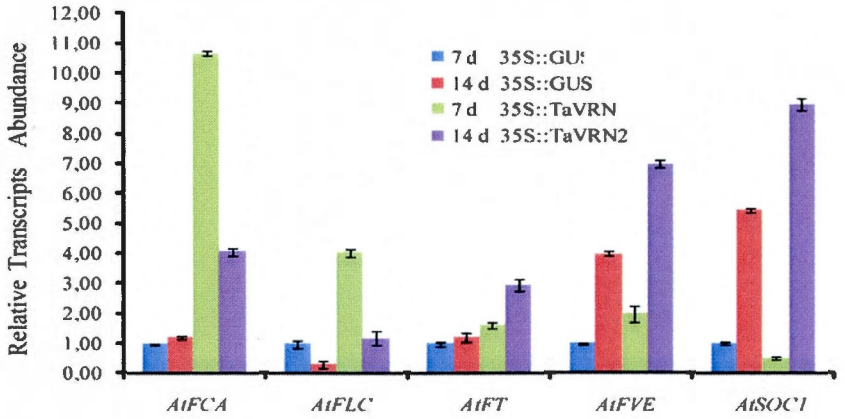


Figure 3.4 Analysis of transgenic *Arabidopsis* plants overexpressing *TaVRN-B2*

A



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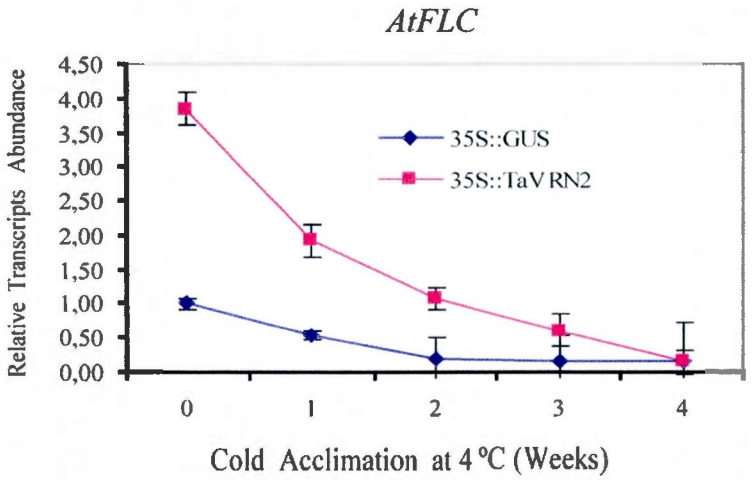


C

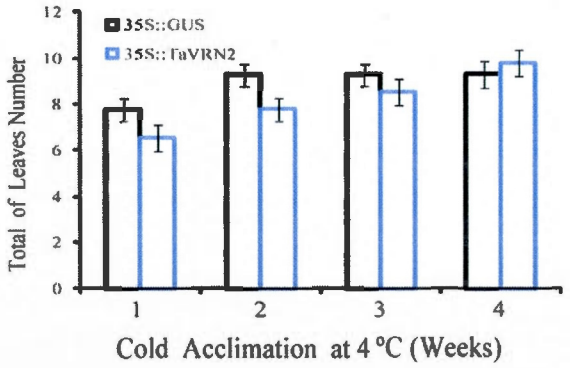
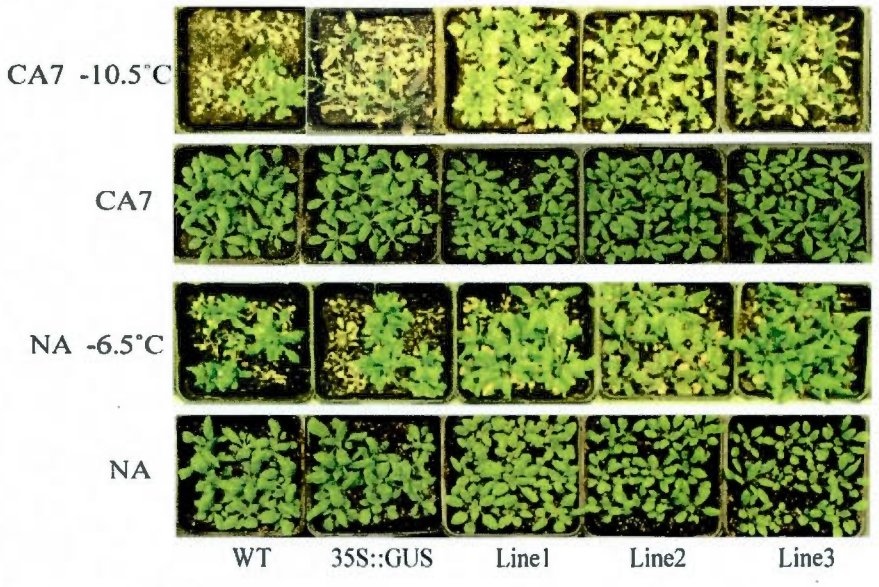


Figure 3.5 Effect of *TaVRN-B2* overexpression on *Arabidopsis* flowering genes

A



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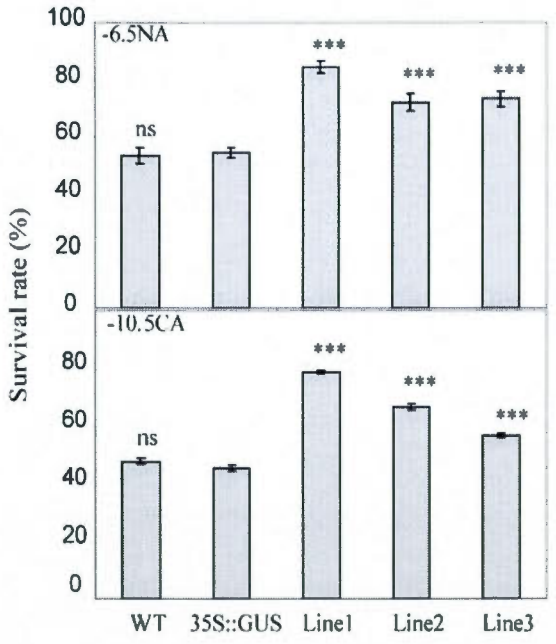

Figure 3.6 *TaVRN-B2* enhances freezing tolerance in *Arabidopsis*

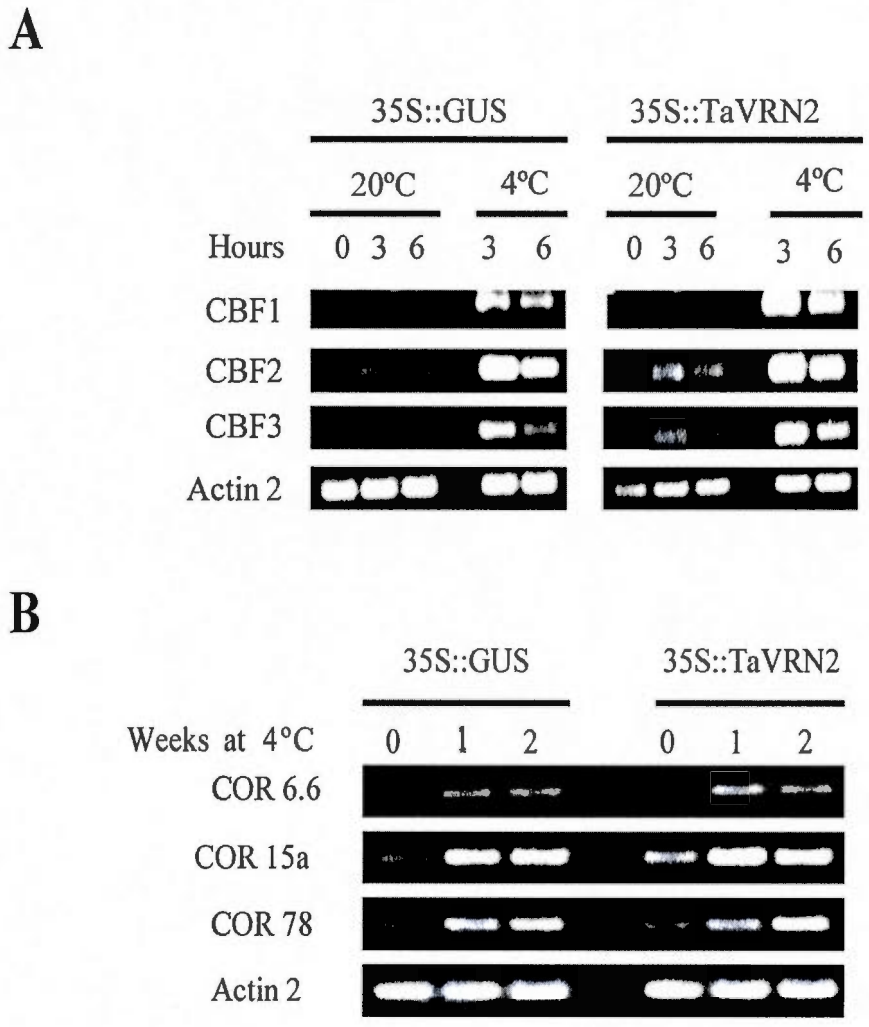

Figure 3. 7 Effect of *TaVRN-B2* over-expression on the accumulation of cold-regulated transcripts

Figure S1: Effect of *TaVRN-B2* over-expression on the accumulation of cold-regulated transcripts

A) Transcript level of *CBFs* genes. Control (*35S::GUS*) and transgenic 35S::*TaVRN-B2* plants (line 1) grown under long day conditions and exposed to 4°C for 3 and 6 hours.

B) Transcript level of *CORs* genes. Control (*35S::GUS*) and transgenic *35S::TaVRN-B2* plants (line 1) grown under long day conditions at 4°C for 1 and 2 weeks. Panels A and B are scanned for densitometry measurement. Relative expression is normalized in relation to the expression of *CBF-2* of transgenic *35S::TaVRN-B2* exposed to 4°C for 3 hours (panel A) and to the expression of *COR 15a* of transgenic *35S::TaVRN-B2* exposed to 4°C for one week (panel B).

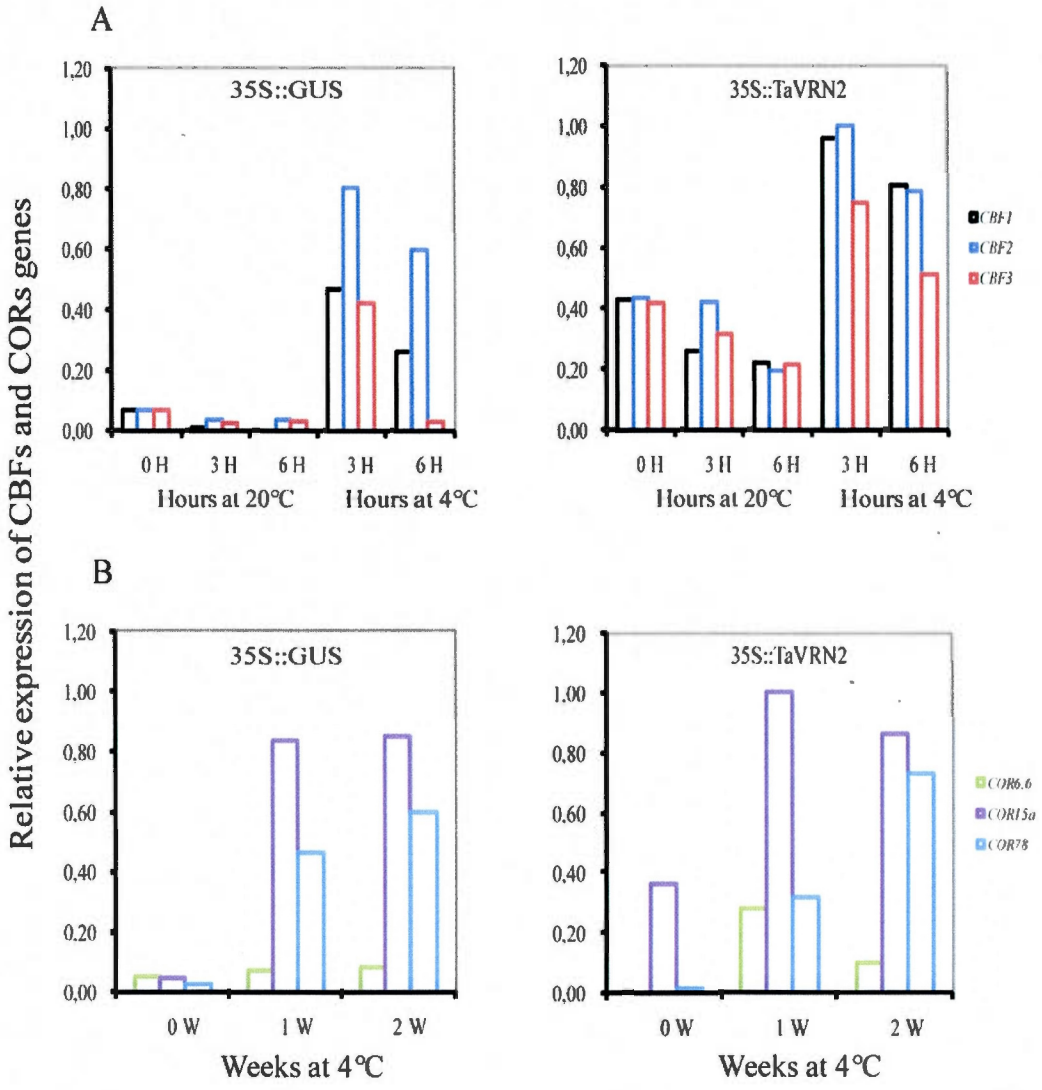

Figure S3.1 Effect of *TaVRN-B2* over-expression on the accumulation of cold-regulated transcripts

Sequences S1: *TaVRN-A2 and TaVRN-B2* open reading frame sequences with specific primers of ZCCT1 highlighted in grey and probe highlighted in yellow.

Genomic sequences of *TaVRN-A2* copy and two promoters from spring wheat cv Manitou and one promoter from winter wheat cv Norstar.

>*TaVRN-A2*, 621pb amplified fron genomic DNA (cv Norstar and cv Manitou), the sequence in obtained after delating the intron.

ATGTCCATGTCATGCGGTTTGTGCGGCGCTAACAACTGCCCGCGCCTCAT
GGTCTCGCCCATTCATCATCGTCATCACCATCATCAGGAGCACCAGCTGC
GTGAGCACCAGTTCTTCGCCCAAGGCAACCACCACCACCACCACCCAGTG
CCACTGCCGCCAGCCAACTTCGACCACAGCAGAACATGGACCACACCATT
TCATGAAACAGCAGCTGCAGGGAACAGCAGCAGGCTCACGCTGGAGGTG
GGCGCAGGCGGCCGACCCATGGCTCACCTAGTGCAGCCACCGGCAAGAG
CCCACATCGTGCCATTTTACGGAGGTGCATTCACCAACACTATTAGCAAT
GAAGCAATCATGACTATTGACACAGAGATGATGGTGGGGCCTGCCCATTA
TCCCACAATGCAGGAGAGAGCAGCGAAGGTGATGAGGTATAGGGAGAAG
AGGAAGAGGCGGCGCTATGACAAGCAAATCCGATATGAGTCCAGAAAAG
CTTACGCTGAGCTTCGGCCATGGGTCAACGGCCGCTTTGTCAAGGTACCC
GAAGCCATGGCGTCGCCATCATCTCCAGCTTCGCCCTATGATCCTAGTAA
ACTTCACCTCGGATGGTTCCGGTAA

>*TaVRN-B2*_ORF_642pb amplified from the winter wheat cold acclimated (cv Norstar) cDNA libraries

ATGTCCATGTCATGCGGTTTGTGCGGCGCCAACAACTGCTCGCGCCTCAT
GGTCTCGCCCATTCATCATCATCATCACCATCATCAGGAGCACCAGCTGC
GTGAGCACCAGTTCTTCGCCCAAGGCAACCACCACCACCACCACCATGGC
GCGGCAGTAGACCACCCAGTGCCACCGCCGCCAGCCAACTTCGACCACCG
CAGAACATGGACTACACCATTTCATGAAACAGCAGCTGCAGGGAACAGC

AGCAGGCTCACACTGGAGGTGGGCGCAGGCGGCCGACACATGGCTCACC
TAGTGCAGCCACCGGCAAGAGCCCACATCGTGCCATTTTATGGAGGTGCA
TTCACAAACACTATTAGCAATGAAGCAATCATGACTATTGACACAGAGAT
GATGGTGGGGCCTGCCCATTATCCCACAATGCAGGAGAGAGCAGCGAAG
GTGATGAGGTATAGGGAGAAGAGGAAGAGGCGGCGCTATGACAAGCAAA
TCCGATACGAGTCCAGAAAAGCTTACGCTGAGCTCCGGCCACGGGTAAAC
GGCCGCTTCGTCAAGGTACCCGAAGCCATGGCGTCGCCATCATCTCCAGC
TTTGCCCTATGGTCCTAGTAAACTTCACCTCGGATGGTTCCGGTAA

***TaVRN2*_genomic_NORSTAR_copy_A_1871pb**

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CCACTGCCGCCAGCCAACTTCGACCACAGCAGAACATGGACCACACCATT
TCATGAAACAGCAGCTGCAGGGAACAGCAGCAGGCTCACGCTGGAGGTG
GGCGCAGGCGGCCGACCCATGGCTCACCTAGTGCAGCCACCGGCAAGAG
CCCACATCGTAAGTAGTAGTACCGCTTAATTGTTTCATCTCTTGCCGATGG
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CATAATTCTGTTAATGTACGCTGCTCAATTGTTCTCCTGAAAAAGATATGC
GGGAATGGATCTTGATATTCTTTAATTTTCTATGGAGGCATATATAGAGTT
TGTGTTTTGTATTAGTTGATGCAGAATTGTATGGGTTGTCAAATCATCAGT
CATACATATAAACTTATTTCATTTTATTTGACCAACAACAAGGTAATCAGT
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GCCATCATCTCCAGCTTCGCCCTATGATCCTAGTAAACTTCACCTCGGATG
GTTCCGGTAA

***TaVRN2*_genomic_MANITOU_ copy_A_1872pb**

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GGTCTCGCCCATTCATCATCGTCATCACCATCATCAGGAGCACCAGCTGC
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CCACTGCCGCCAGCCAACTTCGACCATAGCAGAACATGGACCACACCATT
TCATGAAACAGCAGCTGCAGGGAACAGCAGCAGGCTCACGCTGGAGGTG
GGCGCAGGCGGCCGACCCATGGCTCACCTAGTGCAGCCACCGGCAAGAG
CCCACATCGTAAGTAGTAGTACCGCTTAATTGTTTCATCTCTTGCCGATGG

ATGCGTCCCTGGCTTCCTCCTTAAAAATCCCCACCTAATTTATGTCCATCT
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CATACATGCATACTGAAAATTTGACTTGTGTTCAATAACTAACCAACTCG
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TCAACTTGTCAATATCTATCTGGAGTCCACACTTTATGGTAATTAATTGAC
AAAGTTTTGTGAAATGGACAATATACATACTGGATCGATGCACCCTTTTTC
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TCTTGAGCTAGTTTTGCAAGTCTGTAGCTCATATATAACTGATACTACTCC
CCACGATAGCTTGCGTAGTGGCCGGGTGATCGATCTACCGAGTTCATAAA
ACTGATCGAGATCGGGTCCAAAAAAGAACAAACCCATACAAAATGGAAA
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TTGGCTATCAATCTTTTGAACATGGCATGTTCACCCCAAACGGACTCAGAT
CACAATTATTGATGAAGTTACGCCTTTTAAAAACTCATAAAACTGTACAT
ACATGTACAGGGCTACACACATGTACATAATACACCTAATTAAAACGTAT
ATTCGTAGACCAATTGTTTTGGACGGTGCACATCTTTGAAAAAAAATGCC
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GATACTTCATAAACAGGGGAGTATCAAGTACGTATCTGCTACCCTAAGAA
AGTACATAACTGCGATCTTATGATTATTTTCCTCTTGATGTTCAGGTGCCA
TTTTACGGAGGTGCATTCACCAACACTATTAGCAATGAAGCAATCATGAC
TATTGACACAGAGATGATGGTGGGGCCTGCCCATTATCCCACAATGCAGG
AGAGAGCAGCGAAGGTGATGAGGTATAGGGAGAAGAGGAAGAGGCGGC
GCTATGACAAGCAAATCAGATACGAGTCCAGAAAAGCTTACGCTGAGCTT
CGGCCACGGGTCAACGGCTGCTTTGTCAAGGTACCCGAAGCCATGGCGTC

GCCATCATCTCCAGCTTCGCCCTATGATCCTAGTAAACTTCACCTCGGATG
GTTCCGGTAA

>Promoter TaVRN2 copy 2 from Manitou 760 pb

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CACAGCTGGTAGTACTTTTCTATTTTAGTATTGGCATGGTTTCCATTTTGTT
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TCCGCGTAAGAAAGAAAGAAATCAACGATGGATCGAGGGATCATATCTA
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GTACTCATAACTGCCTCTTCTTCTTCCTCGACATCTCTCCTCCTCGGCCCCT
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AGCTAGCTAGCAGT**ATG**TCCATGTCATGCGGTTTGTGCGGCGCCAACAAC
TGCCCGCGCCTCATGGTCTCGCCCATTCATCATCGTCATCACCATCATCAG
GAGCACCAGCTGCGTCAGCACCAGTTCTTCGCCCAAGGCAACCACCACCA
CC

>Promoter TaVRN2 copy 1 from Manitou 763 pb

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TTCCATTTTGTTGTGTTTGTCTCCCTCGGACCTTTGTGTTAGCATCTCCTTT
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TGTGTGGACATAAGATCATGTGGGGGATTCCCGGCAAGCAAGGTCTGCAT
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GCTGCTAGTACAACTGCCTCTTCTTCTTTCTCGACATCTCTCCTCCTCGGAT
CCTCCACGCACCAGACCACACCAGAAAAAACAAACAAGCAAGCAAACCT
TGGAGCTAGCTAGCAGT**ATG**TCCATGTCATGCGATTTGTGCGGCGCCAAC
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CAGGAGCACCAGCTGCGTGAGCACCAGTTCTTCGCCCAAGGCAACCACCA
CCACC

>Promoter TaVRN2 copy from Norstar 763 pb

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GGACCATATCTATTCCGACCCACTCATTAGTTGGGTCTATTTGATTTGATC
CATTGTATTTTGCTAATTCCCATATCGAATCTTTTTTCTGGCCTTGGAGCTC
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GCTGCTAGTACAACTGCCTCTTCTTCTTTCTCGACATCTCTCCTCCTCGGAT
CCTCCACGCACCAGACCACACCAGAAAAAACAAACAAGCAAGCAAACCT
TGGAGCTAGCTAGCAGT**ATG**TCCATGTCATGCGATTTGTGCGGCGCCAAC
AACTGCTCGCGCCTCATGGTCTCGCCCATTCATCATCATCATCACCATCAT
CAGGAGCACCAGCTGCGTGAGCACCAGTTCTTCGCCCAAGGCAACCACCA
CCACC

Table S3.1 List of primers used in this study

Table S1. Primers used for the different experiments on the characterization of the wheat *TaVRN2* gene.	
Primers used to clone genes and promoters of *TaVRN2*.	
TaVRN2-F	5'-ATGTCCATGTCATGCGGTTTGT-3'
TaVRN2-R	5'-TTACCGGAACCATCCGAGGTGAA 3'
TaVRN2-F2	5'-GTTAAGCTTGGGGGAGAAGAGTCC-3'
TaVRN2-R2	5'-GGTGGTGGTGGTTGCCTTG-3'
Primers used for the generation of construct used to overexpress *TaVRN2* in *Arabidopsis thaliana*. The restriction enzyme cutting sites used for cloning are underlined.	
TaVRN2-F3	5'-CGGATCCTATGTCCATGTCATGCGGTTTGT-3'
TaVRN-R3	5'-AGGAATTCTTTACCGGAACCATCCGAGGTGA-3'
Primers used for qRT-PCR analyses of transcripts expressed in wheat.	
TaVRN-B2/D2-F4	5'-CCGACACATGGCTCACCTAGTG-3'
TaVRN-B2/D2-R4	5'-TTGCTTCATTGCTAATAGTGTTTGT-3'
TaVRN-A2-F5	5'-CGACCCATGGCTCACCTAGT-3'
TaVRN-A2-R5	5'-TTGCTTCATTGCTAATAGTGTTGGT-3'
TaVRN2-PROBE	5'-GGCAAGAGCCCACATCGTGCCATTT-3'
Primers used for RT-PCR analyses of transcripts expressed in *Arabidopsis thaliana* and in wheat.	
AtCBF1-F	5'-TGGAAGCTATTTATACACCGGAAC-3'
AtCBF1-R	5'-GTACAAAAATGGAAACGACTATCGAAT-3'
AtCBF2-F	5'-ACCTTGGTGGAGGCTATTTATACG-3'
AtCBF2-R	5'-CATTTGCATTTGACAACAACTTTTACC-3'
AtCBF3-F	5'-CAGAGCGAAAATGCGTTTTATATGCA-3'
AtCBF3-R	5'-TAATTTACACTCGTTTCTCAGTTTTACA-3'
AtCOR6.6-F	5'-GTGTTAACTTCGTGAAGGACAAG-3'
AtCOR6.6-R	5'-CAAACGTAGTACATCTAAAGGGAGA-3'
AtCOR15a-F	5'-GATACATTGGGTAAAGAAGCTGAGA-3'
AtCOR15a-R	5'-CGGTGACTGTGGATACCATATCTT-3'
AtCOR78-F	5'-GTTGAAGAGTCTCCACAATCACTT-3'
AtCOR78-R	5'-AATCCAATGATTTTACCCACTTTAGAC-3'
AtFCA-F	5'- AATGTACCTGGACCGAGCATACCT-3'
AtFCA-R	5'-CTGCTGAACTTGTTGTGGTTGTTG-3'
AtFLC-F	5'-CGGTTGAAATCAAAATCCAAAACA-3'
AtFLC-R	5'-CACACGAATAAGGTACAAAGTTCATC-3'
AtFVE-F	5'-ATGCAGATACTAACTGGGCACCAA-3'
AtFVE-R	5'-AATCTGTCCCAATCGTTGTGATGT-3'
AtSOC1-F	5'-ACCATAGATCGTTATCTGAGGCAT-3'
AtSOC1-R	5'-GAAGAACAAGGTAACCCAATGAAC-3'
AtFT-F	5'-TAGTAAGCAGAGTTGTTGGAGACG-3'
AtFT-R	5'-GGGAAGGCCGAGATTGTAGAT-3'
Actin2-F	5'-TCAGATGCCCAGAAGTGTGTT-3'
Actin2-R	5'-CCGTACAGATCCTTCCTGATAT-3'
TaVRN2 forward	5'-GGCGGCCGACACATGGCTCA-3'
TaVRN2 reverse	5'-TGGGCAGGCCCCACCATCATC-3'
TaVRT2 forward	5'-GTGGCCGTTGCCGAAGCTGAAAAT-3'
TaVRT2 reverse	5'-CGCGCCATGCAAATGGAGACATAAAACGA-3'
TaVRN1 forward	5'-GCTGAAGGGCTTCCAGCCCATATAAG-3'
TaVRN1 reverse	5'-TACATGGTAAATTGAGCCCAGCTGGG-3'
18S forward	5'-AGTTAAAAAGCTCGTAGTTGGACCT-3'
18S reverse	5'-GTTTATGGTTGAGACTAGGACGGTA-3'

CHAPITRE IV

EXPRESSION OF VERNALIZATION RESPONSIVE GENES IN WHEAT IS ASSOCIATED WITH HISTONE H3 TRIMETHYLATION

Expression of vernalization responsive genes in wheat is associated with histone H3 trimethylation.

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Cet article est en processus de soumission

4.1 RÉSUMÉ

La transition de la phase végétative à la phase reproductive chez le blé d'hiver exige une exposition prolongée aux basses températures, un processus appelé vernalisation. Ce processus est régi par une voie de signalisation génétique impliquant *Triticum aestivum VERNALIZATION1* (*TaVRN1*), *Triticum aestivum VERNALIZATION2* (*TaVRN2*) et *Triticum aestivum FLORWERING LOCUS T* (*TaFT1*) ou *Triticum aestivum VERNALIZATION3*(*TaVRN3*). Ces gènes régulent la floraison en intégrant les signaux environnementaux et l'état de développement de la plante. Pour comprendre le lien entre l'expression de ces gènes et l'état de méthylation de la chromatine lors de la vernalisation chez le blé, le niveau d'expression de deux marqueurs de modifications des histones, le marqueur H3K4me3 un activateur de la transcription et le répresseur H3K27me3 ont été mesurés au niveau des régions promotrices des gènes à l'étude. L'analyse des promoteurs des gènes à partir des espèces céréalières qui exigent la vernalisation révèle la présence d'éléments cis ciblés par des facteurs de transcription communs ce qui suggère leur implication durant la vernalisation en comparaison à des espèces qui n'exigent pas la vernalisation. Ces promoteurs possèdent également des éléments de réponse au Polycomb ou d'éléments de réponse au Trithorax qui lient les groupes de protéines Polycomb et Trithorax, pour maintenir les états de transcription réprimé ou actif des gènes d'importance dans le développement. Des études d'expression de la transcription indiquent que l'expression *TaVRN1* et *TaFT1* chez le blé d'hiver est induite par la vernalisation. Cette régulation à la hausse est associée à une augmentation du niveau de H3K4me3 alors qu'on ne note pas de changement du niveau de H3K27me3 à la région promotrice de *TaVRN1* et *TaFT1*. Cependant pour les deux marqueurs, leur niveau d'expression est maintenu comparable à la région promotrice *TaVRN2*. Cette étude révèle que la transition florale induite par la vernalisation chez le blé d'hiver est médiée par la méthylation des histones au niveau des promoteurs *TaVRN1*, *TaFT1* et T*aVRN2* et peut représenter une partie de la mémoire cellulaire de vernalisation chez le blé.

Mots clés: Chromatine, Épigénétique; Floraison; Histone méthylations; Vernalisation et Blé

4.2 ABSTRACT

The transition to flowering in winter wheat requires prolonged exposure to low temperature, a process called vernalization. This process is regulated by a genetic pathway that involves three genes, *Triticum aestivum VERNALIZATION1* (*TaVRN1*), *Triticum aestivum VERNALIZATION2* (*TaVRN2*) and *Triticum aestivum FLOWERING LOCUS-T like 1* (*TaFT1*). These genes regulate flowering by integrating environmental and developmental cues. To determine whether the expression of these genes is associated with the chromatin methylation state during vernalization in wheat, the level of two markers of histone modifications, the activator histone H3 trimethylation of lysine 4 (H3K4me3) and the repressor histone H3 trimethylation of lysine 27 (H3K27me3) were measured at the promoter regions of these three genes. Bioinformatics analysis of these promoters demonstrates the presence of conserved *cis*-acting elements in the promoters of the three vernalization genes, *TaVRN1, TaVRN2* and *TaFT1*. These elements are targeted by common transcription factors in the vernalization responsive cereals. These promoters also contain the functional "units" PRE/TRE targeted by Polycomb and Trithorax groups of proteins that maintain repressed or active transcription states of developmentally regulated genes. These proteins are known to be associated with the regulation of H3K4me3 and H3K27me3. Expression studies indicate that *TaVRN1* and *TaFT1* in winter wheat are up-regulated by vernalization. This up-regulation is associated with increased level of the activator H3K4me3 with no change in the level of the repressor H3K27me3 at the promoter region of *TaVRN1* and *TaFT1* while the levels of the two types of methylation are implicated at the *TaVRN2* promoter region. This study shows that, the flowering transition induced by vernalization in winter wheat is mediated by histone methylation at the promoter level of *TaVRN1*, *TaFT1* and *TaVRN2* and may represent part of the cellular memory of vernalization in wheat.

Key words: Chromatin; Epigenetic; Flowering; Histone methylations; Vernalization; Wheat

4.3 INTRODUCTION

In temperate cereals, such as wheat and barley and dicot species such as *Arabidopsis*, vernalization allows plants to retain ''memory'' of the prolonged cold of winter, which stimulates flowering when appropriate spring conditions are encountered (Sheldon *et al.*, 2000). In the next sexual generation, the memory of cold is then reset to ensure progeny are competent to respond to vernalization (Trevaskis *et al.*, 2007). This is an example of epigenetic regulation that requires investigation to elucidate the mechanisms involved in that process. Epigenetic regulation of gene expression is mediated in part by post-translational modifications of histone proteins which in turn modulate chromatin structure. Histone modifications are known to influence gene expression (Bernstein *et al.*, 2006). These modifications are relatively stable and are therefore considered potential marks for carrying the epigenetic information through cell divisions (Barski *et al.*, 2007).

Chromatin is divided between silent heterochromatin (condensed) and active euchromatin (extended) forms (Luger *et al.*, 1997). Several post-translationally modified proteins, i.e. methylated, acetylated, phosphorylated, or ubiquitinated, occur in the core histones particularly in regions residing in their N-termini at various amino acid residues (Kouzarides, 2007). In animals as well as in plants, histone modifications play important roles in diverse cellular and developmental processes (Bhaumik, Smith et Shilatifard, 2007 ; Pfluger et Wagner, 2007), and many histone modification sites are conserved between plants and animals (Johnson *et al.*, 2004 ; Zhang *et al.*, 2007). In both plants and animals trimethylation of histone H3 at lysine 27 (H3K27me3) can be established by Polycomb group of proteins and is largely found in inactive gene loci (Kohler et Villar, 2008 ; Schwartz et Pirrotta, 2008). On the other hand, histones H3K4 methylation and acetylation are frequent in the promoter regions of actively transcribed genes (Bernstein *et al.*, 2005 ; Roh *et al.*,

2006). In differentiated cells, with regard to gene activity, H3K4me3 and H3K27me3 are considered as antagonistic modifications (Bernstein *et al.*, 2006). High-resolution profiling of histone methylations in the human genome shows that high level of H3K4me1, H3K4me2 and H3K4me3 are detected surrounding transcription start sites and H3K4 methylations are enriched in promoter regions (Barski *et al.*, 2007).

In *Arabidopsis*, flowering genes involved in floral initiation such as *SUPPRESSOR OF CONSTANS 1 (SOC1)* and *FLOWERING LOCUS T* (*FT*), are repressed by *FLOWERING LOCUS C* (*FLC*), which encodes a MADS-box transcription factor. Vernalization represses *FLC* expression by epigenetic regulation (Helliwell *et al.*, 2006 ; Searle *et al.*, 2006). This epigenetic regulation is associated with increased level of repressive histone modifications including histone H3 lysine 9 dimethylation, histone H4 arginine 3 symmetrical dimethylation, histone H3 lysine 27 di- and trimethylation (H3K27me2, H3K27me3). On the other hand active transcription is associated with modifications such as histone H3 acetylation and histone H3 lysine 4 di- and trimethylation (H3K4me2, H3K4me3) (Bastow *et al.*, 2004 ; Sung et Amasino, 2004 ; Sung *et al.*, 2006). Taken together, these studies suggest that *FLC* chromatin state is modified from an actively transcribed to a repressed form during vernalization treatment. It was shown that the cellular memory of transcriptional repression of *FLC* is maintained during successive cell divisions by mitotic inheritance of repressive histone modifications of the gene (Finnegan et Dennis, 2007). The active *FLC* transcription is restored in progeny, ensuring that the next generation is competent to respond to vernalization (Sheldon *et al.*, 2008). Studies on the molecular mechanisms of vernalization response in *Arabidopsis* raised the question whether the cellular memory and molecular basis of the vernalization response is similar in cereals.

Apart from the genetic definition of some key genes, *VERNALIZATION 1* (*VRN1*), *VERNALIZATION 2* (*VRN2*) and *FLOWERING LOCUS T-like 1 (FT1)*, little is known about the molecular mechanisms regulating the vernalization response in cereals. Winter cultivars of wheat and barley require vernalization to initiate flowering whereas spring cultivars do not. This contrasting response makes winter and spring cereals an excellent model to understand vernalization. Winter cereals respond to seasonal cues, such as temperature and day length by delaying flowering until favourable conditions occur in the spring. This vernalization response is associated with the up-regulation of the floral activator, *TaVRN1* gene (Danyluk *et al.*, 2003 ; Murai *et al.*, 2003 ; Yan *et al.*, 2003). *TaVRN1* encodes a FRUITFULL-like MADS-box transcription factor required for the initiation of reproductive development at the shoot apex (Murai *et al.*, 2003 ; Preston et Kellogg, 2006). The expression of Ta*VRN1* in both winter wheat and barley is induced by vernalization and stay high until the transition to reproductive development stage is reached (Danyluk *et al.*, 2003 ; Murai *et al.*, 2003 ; Yan *et al.*, 2003). *VERNALIZATION 2* (*VRN2*) expression is down-regulated and coincides with the induction of the mobile floral activator *FT1* to accelerate subsequent stages of floral development (Hemming *et al.*, 2008 ; Yan *et al.*, 2004).

FT is known as a floral inducer that encodes a phosphatidylethanolamine binding protein (PEBP) (Kardailsky *et al.*, 1999). In other organisms this class of protein is involved in cellular signalling (Kroslak *et al.*, 2001 ; Yeung *et al.*, 1999). *TaVRN2* is a flowering repressor gene that encodes a zinc finger transcription factor (Diallo *et al.*, 2010 ; Yan *et al.*, 2004). *VRN1* expression level in response to vernalization and its relation with *VRN2* and *FT1* genes shows characteristics of epigenetic regulation, in which *VRN1* is induced by vernalization and its expression is maintained until the transition to reproductive phase and then declines to a low level until the next generation to be reset again by vernalization (Trevaskis *et al.*, 2007 ; Yan *et al.*, 2004). In barley seedlings vernalized under short day conditions,

vernalization caused increased levels of H3K4me3 at exon 1 and the 5' end region of intron 1 of *HvVRN1* and reduced the level H3K27me3 in the promoter measured around -2kb of the transcription start site. These results suggest that vernalization promotes an active state of *HvVRN1* chromatin (Oliver *et al.*, 2009).

This study investigates the regulatory role of histone methylation, in activating or repressing the vernalization associated genes in hexaploid wheat by measuring the levels of two important markers of histone modifications, the activator H3K4me3 and the repressor H3K27me3 within the promoter regions prior to the transcription start site of *TaVRN1*, *TaFT1* and *TaVRN2*. Our data indicates that the flowering transition induced by vernalization in winter wheat is mediated, at least in part, by histone methylation of the chromatin region associated with the proximal promoter region of *TaVRN1*, *TaFT1* and *TaVRN2* chromatin.

4.4 MATERIALS AND METHODS

4.4.1 PLANT MATERIAL AND GROWTH CONDITIONS

Two wheat cultivars (*Triticum aestivum*, 2n x 6 = 42), a spring cultivar Manitou and a winter cultivar Norstar, were grown in a controlled growth chamber as previously described (Diallo *et al.*, 2010). Briefly, plants were grown in a growth chamber at 20°C under long days (LD) (16 hours at 175µmol m^{-2} s^{-1} and 8 hours dark) for 14 days for the non-vernalized plants. For vernalization treatment, 14 days old plants were transferred to 4°C for 63 days under the same LD conditions.

4.4.2 PROMOTER CLONING, ANALYSIS AND PRIMER DESIGN

Promoters of *TaVRN1*, *TaFT1* and *TaVRN2* genes were PCR-amplified from genomic DNA extracted on spring and winter wheat using specific primers designed from sequences obtained from Genbank (http://www.ncbi.nlm.nih.gov/genbank). Primers used to PCR-amplified *TaVRN2* promoter is reported previously (Diallo *et al.*, 2010). Each promoter was analysed to identify the *cis*-elements using PlantCare and PLACE programs. Four regions were identified in each promoter of *TaVRN1*, *TaFT1* and *TaVRN2* and named region 1, 2, 3 and 4. They were generated to determine specific primer sets for qPCR analysis and also to have an amplicon size of PCR product between 150 to 250 bp. The PCR primers were designed with the PRIMER3 program (http:// frodo.wi.mit.edu/primer3/input.htm) with default parameters (oligo size, 20 nucleotides and Tm, 60°C), with an expected 150 to 250 bp product of promoter region. qPCR primer sets are listed in the supporting information section (Table SIII) for *TaFT1*, *TaVRN1* and *TaVRN2*.

4.4.3 IDENTIFICATION OF *CIS*-REGULATORY ELEMENTS IN PROMOTER REGIONS

VRN1 sequences from *Triticum aestivum* (*TaVRN1*-A, *TaVRN1*-B) to *Triticum monococcum* sequence and *VRN1* homologous genes from *Aegilops tauschii*, and *Hordeum vulgare*, *FT* sequences from *Triticum aestivum* and *FT* homologous genes from *Hordeum vulgare*, *Oryza sativa* and *Zea mays* and *VRN2* sequences from *Triticum aestivum*, *Triticum monococcum*, *Triticum turgidum*, and *Hordeum vulgare* were retrieved from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and from *Brachypodium* Genome (http://www.brachybase.org) for the *Brachypodium distachyon* homologs of these genes. mVista Shuffle-LAGAN was used to create pairwise alignments of *VRN1, FT, or VRN2* promoters sequences (http://genome.lbl.gov/vista) (Brudno *et al.*, 2003 ; Frazer *et al.*, 2004). Sequences of conserved regions were analyzed with ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2) (Larkin *et al.*, 2007). Plant *cis*-acting regulatory DNA elements were searched through the PLACE database with a cutoff of >4 bp (http://www.dna.affrc.go.jp/PLACE) (Higo *et al.*, 1999) and Plant-CARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Lescot *et al.*, 2002). The accession numbers of promoters' sequences for all species are listed in supporting information section (Table SII). The sequences of *TaVRN1*, *TaFT1* and *TaVRN2* promoters with their *cis*-acting elements are underlined or highlighted and shown in the supporting information section (Figure S1) and their functions are cited in the same section (Table SI).

4.4.4 RNA ISOLATION AND QUANTITATIVE REAL TIME PCR

Total RNA was isolated from wheat aerial parts at different stages using TRI Reagent (Invitrogen) and three micrograms of total RNA were reverse-transcribed (RT) and subjected to quantitative real-time PCR. RT-PCR analyses were performed using SuperScript™ First-Strand Synthesis System for RT-PCR KIT according to the

manufacturer's instructions (Invitrogen). Transcript level of the genes (*TaVRN1* and *TaFT1* was measured by qRT-PCR using SYBR Green. For *TaVRN2,* the transcript level was measured using the TaqMan method and specific primer sequences (Diallo *et al.*, 2010). TaqMan primer sets were designed according to the specific sequence of the target gene (Invitrogen). For genes expression, we used the copies from *Triticum monococcum* of *TaVRN1* and *TaFT1* (the A copy), the primers used for the expression of *TaFT1* target exclusively *Flowering locus T* and not FT-like genes as previously reported (Shimada *et al.*, 2009) and the B copy of *TaVRN2* and their respective promoters that we cloned. The B copy of *TaVRN2* is clearly associated with vernalization rather than the A copy based on our previous report (Diallo *et al.*, 2010). Each value is the mean of four biological separate qRT-PCR reactions normalized with respect to 18S ribosomal RNA transcript levels. Relative transcript abundance was calculated and normalized with respect to 18S ribosomal RNA. Data shown represent mean values obtained from four biological replicates and the error bars indicate the ± SE of the mean with three independent amplification reactions giving the same results. Amplification efficiency (98% to 100%) for the two primer sets was determined by amplification of cDNA dilution series of 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 ng per reaction (data not shown). Specificity of the qRT-PCR products was assessed by melting curve analysis and gel electrophoresis.

Real-time PCR analysis was performed in a Roche Light Cycler_480 according to the manufacturer's instructions. The program used contains four steps. The first step is the initial activation at 95°C for 15 min. The next step is the PCR amplification performed up to 40 cycles with 95°C for 15 sec, 58°C for 30 sec and 72°C for 30 sec. The third step is the melting curve for 1 cycle (95°C for 5 sec, 65°C for 30 sec and 95°C continuous). The last step of the program is the cooling for 1 cycle at 37°C for 5 min.

To determine the specificity of the primer sets designed for regions 1 through 4 of the *TaVRN1*, *TaFT1* and *TaVRN2* promoter, a serial dilution of 20, 10, 5, 2.5, 1.25 and 0.625 ng per reaction of genomic DNA of winter cultivar Norstar were used for standard curve generation and primer efficiency (E). The ChIP enrichment for H3K4 and H3K27 trimethylation was quantified by comparing the Crossing Point (CP) of the ChIP sample with 18S gene CP of the same sample. The qPCR data are shown as fold enrichment over the control gene and are the means of four independent biological replicates and the error bars indicate the ± SE of the mean. Two technical replicates were made for each sample and gave the same results. Primers used for qRT-PCR and ChIP qPCR are described in the supporting information section (Table SIII). We used the 18S ribosomal RNA gene that is not regulated by vernalization as control and found no change between the vernalized and non-vernalized samples with regard to 18S ribosomal RNA expression in DNA obtained from H3K27Me3 and H3K4Me3 ChIPs.

For real time analysis, we used the mathematical model proposed by Pfaff (Pfaffl, 2001) to assess the relative expression levels of targets genes in comparison to a reference gene. The relative expression ratio (*R*) of target gene is calculated based on efficiency (*E*) and the CP deviation of a treated sample versus a control, and expressed in comparison to reference gene:

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}}\,(control\,-sample)}}{(E_{\text{ref}})^{\Delta CP_{\text{ref}}\,(control\,-sample)}}$$

E_{target} is the PCR efficiency of the target gene transcript; E_{ref} is the PCR efficiency of the reference gene transcript; ΔCP_{target} is the CP deviation of control – sample of the target gene transcript; ΔCP_{ref} is the CP deviation of control – sample of the reference gene transcript.

4.4.5 WESTERN BLOT

Nuclear proteins were pre-cleared with Protein A agarose beads before immuno precipitation step (Supplemental material and methods) and used for western blot analysis. Equal amounts of nuclear proteins were then separated on a 12% SDS–polyacrylamide gel and transferred electrophoretically for 1 hour to a 0.45 μm polyvinylidene fluoride (PVDF) membrane (Life Sciences BioTrace™). The membrane was blocked in a 4% (w/v) solution of reconstituted skimmed milk powder prepared in PBS containing 0.2% (v/v) Tween 20 and then probed with anti-histone H3 (Catalog # ab1791 from abcam Inc. http:// www.abcam.com), anti-acetyl-Histone H3 (Catalog # 06-599) , anti-trimethyl-Histone H3 (Lys4) (Catalog # 07-473) and anti-trimethyl-Histone H3 (Lys27) (Catalog # 07-449) from Millipore, http:// www.millipore.com/) at a 1:5,000 dilution overnight. After washing with PBS–Tween 20, the proteins recognized by the primary antibody were revealed with a goat anti-rabbit HRP conjugated IgG (Catalog # 12-348) at a 1:20,000 dilution. The complexes were visualized using the enhanced chemiluminescent detection system using the HyGLO™ Chemiluminescent HRP Antibody Detection Reagent) and HyBlot CL™ Autoradiography Film (DENVILLE Scientific Inc. P.O. Box 4588 Metuchen, NJ 08840-4588 USA, www.denvillescientific.com).

4.4.6 CHROMATIN IMMUNOPRECIPITATION (ChIP)

Chromatin immunoprecipitation was performed essentially as described in detail in the supporting information section. Chromatin from plants at various stages of development was sonicated with a Sonic Dismembrator Model 500 (Fisher Scientific) for 1 min with a 40% duty cycle two times. Chromatin was precipitated with antibodies anti-trimethyl-Histone H3 (Lys4) and anti-trimethyl-Histone H3 (Lys27). After reverse cross-linking and proteinase K treatment. DNA was extracted with 1 volume of phenol-chloroform and precipitated with 1/10 volume 3M NaOAc pH5.2

and 3 volumes absolute ethanol. The resulting DNA was analysed by quantitative real-time PCR.

4.5 RESULTS

4.5.1 EXPRESSION LEVEL OF *TaVRN1, TaFT1* AND *TaVRN2* IN RESPONSE TO VERNALIZATION

In nature, vernalization normally occurs under short day (SD) conditions where *TaFT1* and *TaVRN2* expression are down-regulated (Beales *et al.*, 2007 ; Diallo *et al.*, 2010). On the other hand, spring wheat used as control is grown under LD conditions to promote flowering. Previous studies have shown that vernalization of winter wheat is as efficient under SD or LD conditions in wheat (Fowler *et al.*, 1996 ; Kane *et al.*, 2005). The rationale of using spring wheat under LD conditions that does not require vernalization as control is to neutralize the effect of both cold and day length across the experiment and to compare the expression behaviour of the three genes in closely related genotypes that differ in vernalization requirement. *TaVRN2* and *TaFT1* are known to be repressed by short day in spring wheat. Under short day, we would have the problem to differentiate the effect of vernalization response and the photoperiod response with regard to these two genes and under short day, we could not use spring wheat as control. We used the same light regime for vernalization of winter wheat to neutralize the effect of the photoperiod and allow the comparison between the expression level of the three genes and the H3K4me3 and the H3K27me3 markers in both spring and winter wheats.

Our choice of the experimental design was based on our previous work using wheat, where we demonstrated that the day length does not affect the vernalization response in winter wheat as determined by the leaf number and the double ridge as previously described (Danyluk *et al.*, 2003 ; Fowler *et al.*, 1996 ; Kane *et al.*, 2007 ; Kane *et al.*, 2005). The focus of this paper is to determine the impact of histone methylation on the vernalization responsive genes in vernalized and non-vernalized winter wheat using flowering competent spring wheat as reference. The appearance

of double ridges in winter wheat, cv Norstar (arrow in Fig. 1) is clearly associated with the up-regulation of *TaVRN1* and *TaFT1* and the repression of *TaVRN2* (Fig. 2). In spring wheat, cv Manitou, the vernalization treatment shows a highly developed shoot apex (Fig.1) that is associated with the down regulation of *TaVRN1* and *TaFT1* mRNA expression while no significant changes was seen in *TaVRN2* mRNA expression level that is present in very low abundance in the spring wheat (Fig. 2). These results demonstrate that in spring wheat, plants have the competence to flower before vernalization treatment in contrast to winter wheat that requires vernalization to acquire competence to flower.

Using vernalized and non-vernalized winter and spring wheat, we investigated two of the histone modifications markers that occur in response to vernalization at the level of the promoter of the three vernalization responsive genes, *TaVRN1*, *TaFT1* and *TaVRN2*. Preliminary analysis by western blotting, using antibodies against three histone modification markers, anti-acetyl-Histone H3, anti-trimethyl-Histone H3 (Lys4) (or anti-H3K4me3) and anti-trimethyl-Histone H3 (Lys27) (or anti-H3K27me3) indicate that only H3K4me3 and H3K27me3 markers accumulate to a higher level in response to low temperature treatment in both spring and winter wheat nuclear proteins compared to anti-Histone 3 (anti-H3) used as load control (Fig. 3). The acetyl-Histone H3 showed no signal in both spring and winter wheat in response to low temperature treatment (data not shown). These results suggest that the changes in the methylation status of the two histone modification markers H3K4me3 and H3K27me3 are associated with chromatin modifications induced by low temperature. To investigate the effect of these modifications on gene expression, we performed ChIP experiments focusing on the three vernalization associated genes, *TaVRN1*, *TaFT1* and *TaVRN2*. DNA content in the chromatin extract was quantified to ensure that equal amounts of DNA were used in these experiments.

To determine if gene expression is associated with changes in the chromatin methylation state at each gene promoter region, we first hypothesized that the regulation of these genes at the promoter level could be located in the promoter region that contains the *cis*-regulatory elements involved in the transcriptional regulation of gene activities controlling various biological processes, including abiotic stress responses, hormone responses and developmental processes (Yamaguchi-Shinozaki et Shinozaki, 2005).

4.5.2 IDENTIFICATION OF THE CIS-ACTING ELEMENTS AND PAIRWISE ALIGNMENT IN THE PROXIMAL *VRN1*, *FT1* AND *VRN2* PROMOTERS

To identify the genomic target location associated with increased or decreased H3K4me3 and H3K27me3 methylation, the promoter region of *TaVRN1* and *TaFT*1 were cloned from genomic DNA of both winter wheat cv Norstar and spring wheat cv Manitou. The sequences were analysed to identify *cis*-regulatory elements (Fig. S1). *TaVRN2* promoter was previously cloned (Diallo *et al.*, 2010). The mVISTA Shuffle-LAGAN was used to analyze the upstream sequences of the transcription start of *VRN1*, *FT* and *VRN2* respectively from wheat and other species (Fig. 4). The pairwise alignment of the targeted 1kb *VRN1* promoter region from *Triticum aestivum* (*TaVRN-A1*, *TaVRN-B1*), *Triticum monococcum*, *Aegilops tauschii*, *Hordeum vulgare* and *Brachypodium distachyon* revealed highly conserved sequences (Fig. 4a and Fig. 5). A similar observation was also found with *VRN2*. The pairwise alignment revealed highly conserved sequence in the first 627 bp upstream of *VRN2* start codon (Fig. 4b). The high conservation sequence is very clear between *T. aestivum, T. monococcum, T. turgidum H. vulgare*, and less with *B. distachyon* (Fig. 4b and Fig. 6). The alignment shows several documented transcription factor binding sites (Table SI; (Diallo *et al.*, 2010)). *FT*, a gene that is present in both vernalization responsive species (wheat, barley, *Brachypodium* and *Arabidopsis*) and vernalization insensitive

ones (rice, maize) is a good example to determine whether differences can be identified between promoters of vernalization sensitive and insensitive species. The pairwise alignment reveals high sequence conservation in the first 900 bp upstream of *FT* start codon between *Triticum aestivum* and *Hordeum vulgare*, while less significant sequence conservation was observed with *Brachypodium distachyon*. No sequence conservation was found within FT promoters from *Oryza sativa* and *Zea mays* (Fig. 4b and Fig. 6). Promoter sequences conservation is important between the vernalization sensitive species, wheat and barley, while no conservation can be found with the sequence of the insensitive species rice and corn. The rationale of using rice and corn in the comparison is to make the evolutionary point that the conserved flowering gene *FT* is different in species that require vernalization, and that this difference may be due to the evolution of flowering plant that adapted to temperate climates. This suggests that wheat and barley promoter sequences contain conserved regulatory elements associated with the vernalization response. To verify this hypothesis, we used PLACE and PlantCare programs to analyse promoter motifs.

To identify and compare the putative c*is*-regulatory elements of the flowering genes *FT*, *VRN1* and *VRN2* from vernalization sensitive and insensitive species, PlantCARE and PLACE database were used. Results show highly conserved putative *cis*-regulatory elements between wheat species and barley. Several *cis*-elements involved in light responsiveness, endosperm specific gene expression, hormone responsiveness, meristem specific expression and stress responsiveness were found to be distributed in the promoters of these genes in species that required vernalization (Table SI). In addition, the CCAAT box, the binding sites for the NF-Y complex (Mantovani, 1999) are found in the three promoters (Figs. 5-7) and (Table SI). Promoter analysis also reveals that *TaVRN1* and *TaFT1* have two C-box (CACGTC) and one G-box (CACGTG) respectively. Theses domains are targeted by bZIP proteins. All three promoters, *TaVRN1*, *TaFT1* and *TaVRN2* contain motif sites (ACGT) recognized by MyoD proteins member of bHLH transcription factor proteins

and GATA1 proteins (Fig. S1 and Table SI). It is hypothesized that these transcription factors could be involved in the vernalization response (Golovnina *et al.*, 2010). Furthermore, the region surrounding the CArG-box domain of *TaVRN1* and *TaFT1* promoter include a recognition site for HMG1 protein (high mobility group protein) suggested to participate in modifying chromatin structure by interacting with histones (Golovnina *et al.*, 2010).

The analysis also showed the presence of PcG and TrxG functional "units" in the promoter of *FT*, *VRN1* and *VRN2* genes (Fig. S1 and Table SI). PcG and TrxG proteins bind to these functional "units" referred as Polycomb response elements (PRE) or Trithorax response elements (TRE), and often these bi-functional "units" are called 'cellular memory modules' (Ringrose et Paro, 2004). These c*is*-acting components are suggested to be regulatory DNA sequences in the vernalization response (Sung et Amasino, 2006). The c*is*-regulatory elements, the functional "units" and their putative functions are shown in the supporting information section (Table SI). Promoter analysis reveals the presence of functional "units" PRE/TRE targeted by Polycomb and Trithorax involved in H3K4me3 and H3K27me3 levels. It is likely that these complexes are involved in the addition and maintenance of H3K4me3 and H3K27me3 at *TaVRN1, TaFT1 and TaVRN2*.

Taken together, these results suggest that the promoter that contains conserved *cis*-acting elements associated with vernalization response contain elements that are known to participate in the regulation of higher order chromatin structure through epigenetic modifications. Chromatin modifications may thus be involved in regulating the expression of the vernalization responsive genes in wheat. To determine the relationship between gene expression and changes in the chromatin methylation state at each gene promoter region, chromatin immunoprecipitation (ChIP) experiments were performed using chromatin obtained from vernalized and

non-vernalized plants from both spring and winter wheat. For each promoter we defined four regions that we named 1, 2, 3 and 4 as described in figures 8a-10a.

4.5.3 *TaVRN1* AND *TaFT1* UP-REGULATION IS ASSOCIATED WITH INCREASED HISTONE METHYLATION

The relative abundance of two histone modification markers, H3K4me3, a modification associated with active gene transcription, and H3K27me3, a modification associated with gene repression (Barski *et al.*, 2007 ; Roh *et al.*, 2006) was measured at *TaVRN1* and *TaFT1* chromatin in the vernalized and non-vernalized hexaploid winter wheat cv Norstar and spring wheat cv Manitou. *TaVRN1*, *TaVRN2* and *TaFT1* promoters sequence analysis of approximately 1 kbp genomic DNA fragment upstream of the 5'UTR indicated that there are few polymorphisms between the different cultivars that differ in their vernalization requirements. A 17 bp deletion is found only in the promoter from the spring cultivar Manitou of *TaVRN1* promoter sequence but this deletion is not found in the promoters of other spring wheat varieties (cultivars Glenlea, Concorde), or from winter wheat varieties (cultivars Cheyenne, Absolvent, Norstar). This result suggests that this indel is not involved in the lack of vernalization requirement in spring wheats (Kane *et al.*, 2007). For *TaVRN1,* the promoter region from - 860 to + 3, corresponding to the ATG was used. The *TaVRN1* promoter primer sets of region 3 and 4 were generated by avoiding the CArG box sequence and the indel of the cv Manitou promoter.

For *TaFT1*, the promoter region from - 897 to -143 was used. The four regions of the promoter of *TaVRN1* (Fig. 8a) and *TaFT1* (Fig. 9a) were analyzed in ChIP experiments using antibodies directed against H3K4me3 or H3K27me3. The results indicate that in winter wheat, vernalization caused an enrichment in the level of H3K4me3 at region 1, 2 and 4 of *TaVRN1* promoter (Fig. 8b) while no significant changes were seen in the level H3K27me3 at the four regions of the same promoter

(Fig. 8c). In spring wheat vernalization caused a decrease in the level of H3K4me3 at regions 1 and 4 while no significant changes were seen in regions 2 and region 3 (Fig. 8d). H3K27me3 level did not exhibit significant changes in spring wheat after vernalization treatment (Fig. 8e). The increase in H3K4me3 level with no significant changes in H3K27me3 at *TaVRN1* suggest that vernalization promotes an active state of *TaVRN1* chromatin in winter wheat and the decrease in H3K4me3 in spring wheat suggest that vernalization reduced the level of active state of *TaVRN1* chromatin. These results are consistent with the relative abundance of *TaVRN-A1* mRNA in response to vernalization in winter and spring wheat (Fig. 2). Region 4 of *TaVRN1* promoter, the closest to the start codon is associated with the most important changes of H3K4me3 level in both winter and spring wheat in response to vernalization treatment and the developmental stages (Fig. 8b and Fig. 8d). Vernalization treatment did not affect the relative abundance of H3K27me3 in winter as well in spring wheat (Fig. 8c; and Fig. 8e). Vernalization caused also an increased level of H3K4me3 methylation at *TaFT1* promoter regions 1, 3 and 4 (Fig. 9b) in winter wheat while no significant changes were seen in the level H3K27me3 at the four regions of the promoter (Fig. 9c). On the other hand, in spring wheat vernalization caused a decrease in H3K4me3 level at regions 2, 3 and 4 while no significant changes were observed in region 1 (Fig. 9d).

The level of H3K27me3 shows also a decrease in spring wheat after vernalization treatment (Fig. 9e). The increased level in H3K4me3 coupled by the unchanged level in H3K27me3 at *TaFT1* suggests that vernalization promotes an active state of *TaFT1* chromatin in winter wheat. The decrease in both H3K4me3 and H3K27me3 levels in spring wheat is more difficult to interpret but it is known (from Fig. 1) that the level of *TaFT1* mRNA drops in spring wheat after vernalization when the meristem may be fully committed to flowering. The expression of TaFT1 proteins is not required any more, even if environmental conditions may be permissive and other developmental factors may affect the activity of this chromatin region. Together

these results indicate that vernalization reduced the level of active state of *TaFT1* chromatin in spring wheat. This interpretation of the results is in agreement with the relative abundance of *TaFT1* in response to vernalization in winter and spring wheat (Fig. 2).

4.5.4 *TaVRN2* REPRESSION IS ASSOCIATED WITH INCREASED OF H3K4ME3 METHYLATION NEUTRALIZED BY H3K27ME3 LEVEL IN RESPONSE TO VERNALIZATION

TaVRN2 promoter portion from (- 605 to - 92) was also separated into four regions (Fig. 10a) and the relative abundance level of H3K4me3 and H3K27me3 was measured at *TaVRN2* chromatin in winter Norstar and spring Manitou. The results show that, before vernalization treatment *TaVRN2* chromatin state was associated by an enrichment of H3K27me3 at region 4 in winter wheat. In winter wheat, vernalization caused an increase in the level of H3K4me3 at regions 1, 3 and 4 and no significant change was seen at region 2. We also observed a similar level of increase of H3K27me3 at regions 1 and 3 with no significant change at region 2 and 4. In spring wheat vernalization caused an increased of H3K4me3 at region 1 without affecting other regions (Fig. 10b and Fig. 10d) and no significant changes were seen in the level H3K27me3 at the four regions of the promoter (Fig. 10c and Fig. 10e). The levels of H3K27me3 in the promoter are probably having a greater effect to repress gene expression compared to the action of H3K4me3 (Fig. 10) since the level of *TaVRN2* RNA expression is decreased after vernalization in winter wheat Norstar (Fig. 2). However, these results do not exclude that other histone modifications may be involved in *TaVRN2* regulation.

In this report, our results clearly indicate that both *TaVRN1* and *TaFT1* expressions in response to vernalization are associated to the increase of H3K4me3 with no change with H3K27Me3 in winter wheat. In the case of *TaVRN2*, we suggest that the expression of H3K27Me3 and H3K4Me3 expressions are poised in VRN2 locus with the possibility that some marks at specific positions may have a greater effect.

4.6 DISCUSSION

In this study, we investigated the relationship between gene expression and the chromatin methylation state at the level of the promoter region of the three key vernalization genes, *TaVRN1*, *TaFT1* and *TaVRN2* in both spring and winter wheat. Promoter regions of *TaVRN1*, *TaFT1* and *TaVRN2* that are conserved in vernalization sensitive species contain *cis*-acting elements targeted by proteins involved in vernalization response. Our results demonstrate that the up-regulation of *TaVRN1* and *TaFT1* by vernalization is associated with changes in histone methylation in different regions of the promoters of *TaVRN1* and *TaFT1*. Vernalization treatment causes increased levels of H3K4me3 in *TaVRN1* and *TaFT1* chromatin without change in the H3K27me3 in winter wheat, indicating that vernalization promotes an active state of *TaVRN1* and *TaFT1* chromatin and the memory of vernalization involves epigenetic inheritance of histone modifications associated with active transcription of these two genes.

Analysis of histone modifications at the *VRN-1* locus in barley has shown that the induction of *HvVRN1* is associated with an increase in H3K4me3, and a decrease in H3K27me3 in response to vernalization. This modification was found within the exon1 (region 2) and the 5' end of the first intron. They also found an enrichment of H3K27me3 at the promoter level before vernalization (Oliver *et al.*, 2009). However, this group did not find any histone modifications at the level of *HvVRN2* and *HvFT* (Oliver *et al.*, 2009). In contrast, our findings demonstrate that in wheat, the expression of *TaVRN1* and *TaFT1* is associated with increased H3K4me3 and no changes on H3K27me3 modification at the promoter level while reduced expression of *TaVRN2* may be associated with an increased level of H3K27me3.

H3K4me3 and H3K27me3 are found to colocalize in some genomic regions, although they are enriched at active and inactive chromatin regions respectively (Bernstein *et al.*, 2006 ; Roh *et al.*, 2006). Large scale investigation of H3K4me3 and H3K27me3 in the human genome (Barski *et al.*, 2007) and T cells (Roh *et al.*, 2006 ; Wei *et al.*, 2009) show that active and inactive regions are associated with both H3K4me3 and H3K27me3 modification markers. This is consistent with the fact that genes are not permanently repressed but instead are poised for expression (Barski *et al.*, 2007 ; Wei *et al.*, 2009). These bivalent modifications are identified in more than 3000 promoters in differentiated T cells (Roh *et al.*, 2006). Bivalent domains, where H3K4me3 and H3K27me3 signals exist next to each other, are suggested to play regulatory roles for the differentiation of embryonic stem cells (Bernstein *et al.*, 2006).

H3K4me3 and H3K27me3 levels achieve their action in plants and other organisms through TrxG and PcG protein complexes respectively (Hanson *et al.*, 1999 ; Pien *et al.*, 2008). In cereals such putative core TrxG and PcG complex components were identified (Fu, Dunbar et Dubcovsky, 2007 ; Springer *et al.*, 2003). Because of its presence in the *Drosophila* proteins *Su(var)* (Schumacher et Magnuson, 1997), *Enhancer of zeste*, and *trx* (S) (Jones et Gelbart, 1993), the carboxy-terminal domain termed the SET domain are considered to be in an evolutionarily conserved family of proteins that maintain specific patterns of gene expression after the initiation of transcription during development. The Trithorax (Trx-G) and Polycomb (Pc-G) groups constitute the two major families of SET-containing proteins which are chromatin-associated proteins that act antagonistically to alter chromatin structure to either promote or repress transcription, respectively (Chinwalla, Jane et Harte, 1995 ; Hanson *et al.*, 1999).

In summary, the PcG and TrxG maintain the status of their target genes through mitosis in a manner that is stable but not static. The interaction of PcG and

TrxG proteins with PREs "units" ensures not only a memory of transcriptional history, but also a capacity to switch if a new transcriptional stimulus is received. In principle, this dynamic balance should allow the PcG and TrxG to maintain transcription not only in black and white "on" or "off" states, but also every shades of gray in between (Ringrose et Paro, 2004). It is likely that these complexes are involved in maintaining the appropriate ratio of H3K4me3 and H3K27me3 in *TaVRN1*, *TaFT1* and *TaVRN2* for proper regulation of the vernalization response. This may be mediated by PcG complexes that require PcG-binding and TrxG-binding elements within the genes. The enrichment of H3K4me3 at *TaVRN1* and *TaFT1* chromatin may involve TrxG groups to promote transcription. The enrichment of H3K4me3 at *TaVRN1* and *TaFT1* by vernalization may result from histone methylase activity.

In this study, we investigated the relationship between gene expression and the methylation state of chromatin at the level of the promoter region of the three key vernalization genes, *TaVRN1*, *TaFT1* and *TaVRN2* in both spring and winter wheat. Our results demonstrate that the induction of *TaVRN1* and *TaFT1* expression level by vernalization involves changes in histone methylation at *TaVRN1* and *TaFT1*. Our data on the molecular mechanisms of vernalization in wheat suggest that the vernalization responsive genes are regulated, at least in part, by histone methylation in wheat. Our study demonstrates that chromatin modification is an important part of the regulatory mechanisms that governs the vernalization genes in cereal and opens the way to understand the cellular memory of plant. The complex interplay between activating and repressive inputs that regulate these three vernalization genes at the histone level may regulate the transition from vegetative to reproductive stage and could be part of the cellular memory of vernalization in temperate cereals.

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Author Contributions

AOD and FS conceived and designed the experiments.

AOD, MH and FS wrote the manuscript.

AOD performed the experiments.

MAB-A conducts the qPCR data analysis with AOD and FS and critically read the manuscript.

MB conducts the promoter analysis with AOD and FS and critically read the manuscript.

Short legends for Supporting Information

Figure S1: *TaVRN1*, *TaFT* and *TaVRN2* promoter analysis

Table SI: Functions of identified *cis*-regulatory elements in the promoters of genes

Table SII: Accession numbers of promoter sequences

Table SIII: Primers used in this study

Detailed Chromatin Immuno Precipitation (ChIP) protocol is presented

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Figure legends

Figure 1: Apex development in vernalized and non-vernalized winter and spring wheat. After 2 weeks of germination at 20°C under long day photoperiod, non-vernalized, spring (cv Manitou) and winter (cv Norstar) wheat plants were then vernalized under a long day photoperiod at 4°C for 63 days. The main stem shoot apices were dissected and photographed. The arrow indicates the double ridge. Scale bars are indicated.

Figure 2: Relative expression levels of *TaVRN1*, *TaFT1* and *TaVRN2* during vernalization in hexaploid wheat seedling analysed by quantitative real-time PCR.
After 2 weeks of germination at 20°C under long day photoperiod, non-vernalized (NV), spring (cv Manitou) and winter (cv Norstar) wheat plants were vernalized (V) under a long day photoperiod at 4°C for 63 days. The level of expression in each panel is calibrated by the non-vernalized point. Data represents the mean ± SEM from 4 biological replicates.Three technical replicates gave the same results. The levels are normalized by *Ta 18S RNA*.

Figure 3: Immunoblot analysis of nuclear protein extracted from aerial plant tissues.
Proteins were obtained from wheat plants germinated for 2 weeks at 20°C under long day photoperiod for non-vernalized (NV) spring (cv Manitou or Man) and winter (cv Norstar or Nor) wheat or kept under a long day photoperiod at 4°C for 63 days for vernalized (V) plants. Equal amounts of nuclear protein extracts pre-cleared with Protein A agarose beads from the immuno-precipitation step in the ChIP protocol were separated by SDS–PAGE and transferred to a polyvinylidene difluoride membrane, and then probed with an anti-histone H3, anti-H3K4me3 or anti-H3K27me3 antibodies. The anti-histone H3 was used as load control. The experiment was repeated with 4 different biological replicates showing the same result.

Figure 4: Pairwise alignment of *VRN1, FT and VRN2* promoter sequences from different species using mVISTA Shuffle-LAGAN program.
1 kbp of *VRN1* promoter sequence of *Triticum aestivum, (TaVRN-A1, TaVRN-B1), Triticum monococcum, Hordeum vulgare, Aegilops tauschii* and *Brachypodium distachyon (a),* 1.6 kb p of *FT* promoter sequence of *Triticum aestivum, Triticum tauschii, Hordeum vulgare, Brachypodium distachyon, Oryza sativa, Zea mays* and *Arabidopsis thaliana (b)* and 0.6-kb *VRN2* promoter sequence of *Triticum aestivum, Triticum monococcum, Triticum turgidum, Hordeum vulgare* and *Brachypodium distachyon* (c) were used for these alignments. Graphical output shows base pair similarity between sequences of the different species and *TaVRN1*, *TaFT*or *TaVRN2*. For accession numbers, see Table SII.

Figure 5: Pairwise alignment of *VRN1* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2.
1 kbp of *VRN1* promoter sequence of *Triticum aestivum, (TaVRN-A1, TaVRN-B1), Triticum monococcum, Hordeum vulgare, Aegilops tauschii* and *Brachypodium distachyon*

Figure 6: Pairwise alignment of *FT* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2.
1 kbp of *FT* promoter sequence of *Triticum aestivum, Triticum tauschii, Hordeum vulgare, Brachypodium distachyon, Oryza sativa, Zea mays* and *Arabidopsis thaliana.*

Figure 7: Pairwise alignment of *VRN2* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2.
0.6-kbp *VRN2* promoter sequence of *Triticum aestivum, Triticum monococcum, Triticum turgidum, Hordeum vulgare* and *Brachypodium distachyon*

Figure 8: Effect of vernalization on histone modifications at different *TaVRN1* promoter regions in hexaploid wheat seedlings. (**a**) Diagram of the *TaVRN1* promoter showing the regions (1 - 4 dashed lines) analysed by ChIP, followed by quantitative real-time PCR. Relative abundance of H3K4me3 (**b** and **d**) and H3K27me3 (**c** and **e**) at *TaVRN1* in non-vernalized (NV) and vernalized (V) seedlings from cv Norstar (**b** and **c**) and cv Manitou (**d** and **e**), normalized by *Ta18S* gene. In each panel, the NV point in region 1 is used as a calibrator. Data represents the mean ± SEM from 4 biological replicates. The threshold for statistical significance difference between NV and V is: *: $p < 0.05$; **: $p < 0.01$.

Figure 9: Effect of vernalization on histone modifications at different *TaFT1* promoter regions in hexaploid wheat seedlings. (**a**) Diagram of the *TaFT1* promoter showing the regions (1 - 4 dashed lines) analysed by ChIP, followed by quantitative real-time PCR. Relative abundance of H3K4me3 (**b** and **d**) and H3K27me3 (**c** and **e**) at *TaFT1* in non-vernalized (NV) and vernalized (V) seedlings from cv Norstar (**b** and **c**) and cv Manitou (**d** and **e**), normalized by *Ta18S* gene. In each panel, the NV point in region 1 is used as a calibrator. Data represents the mean ± SEM from 4 biological replicates. The threshold for statistical significance difference between NV and V is: *: $p < 0.05$; **: $p < 0.01$.

Figure 10: Effect of vernalization on histone modifications at different *TaVRN2* promoter regions in hexaploid wheat seedlings. (**a**) Diagram of the *TaVRN2* promoter showing the regions (1 - 4 dashed lines) analysed by ChIP, followed by quantitative real-time PCR. Relative abundance of H3K4me3 (**b** and **d**) and H3K27me3 (**c** and **e**) at *TaVRN2* in non-vernalized (NV) and vernalized (V) seedlings from cv Norstar (**b** and **c**) and cv Manitou (**d** and **e**), normalized by *Ta18S* gene. In each panel, the NV point in region 1 is used as a calibrator. Data represents the mean

± SEM from 4 biological replicates. The threshold for statistical significance difference between NV and V is: *: $p < 0.05$.

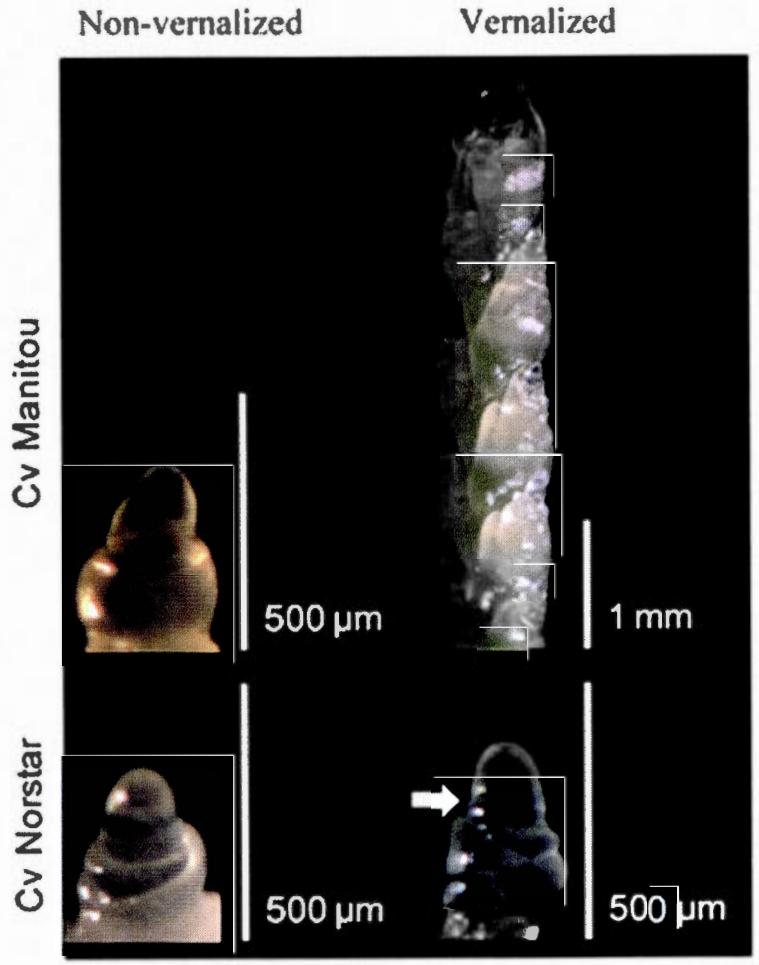

Figure 4.1 Apex development in vernalized and non-vernalized winter and spring wheat

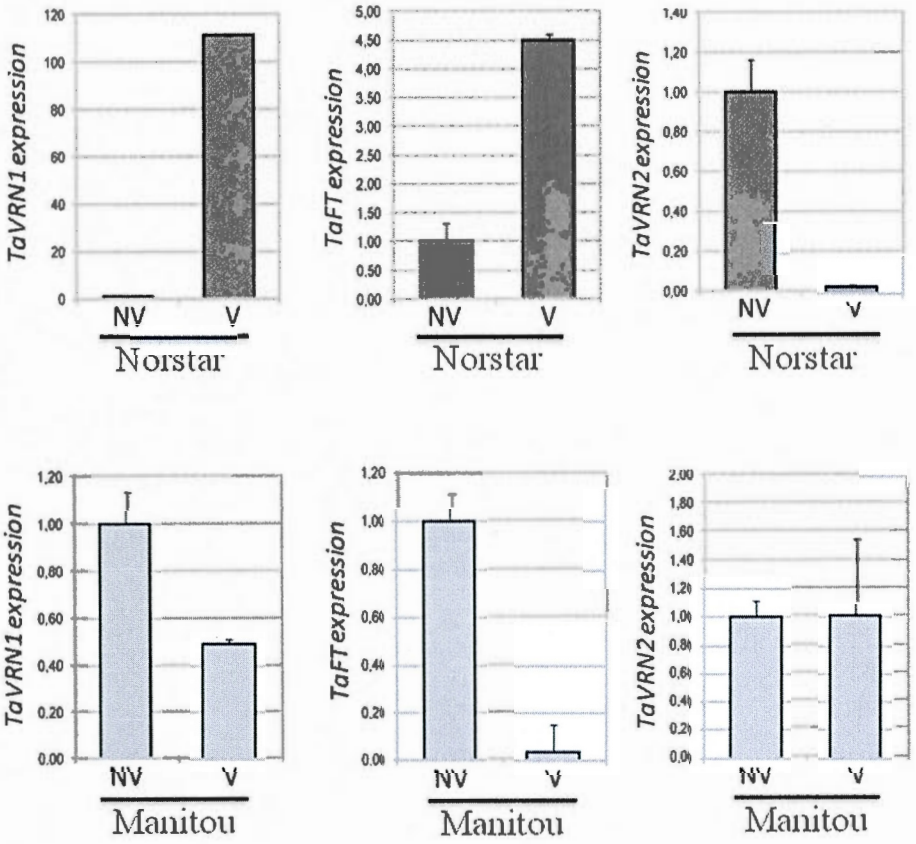

Figure 4.2 Relative expression levels of *TaVRN1*, *TaFT1* and *TaVRN2* during vernalization in hexaploid wheat seedling analysed by quantitative real-time PCR

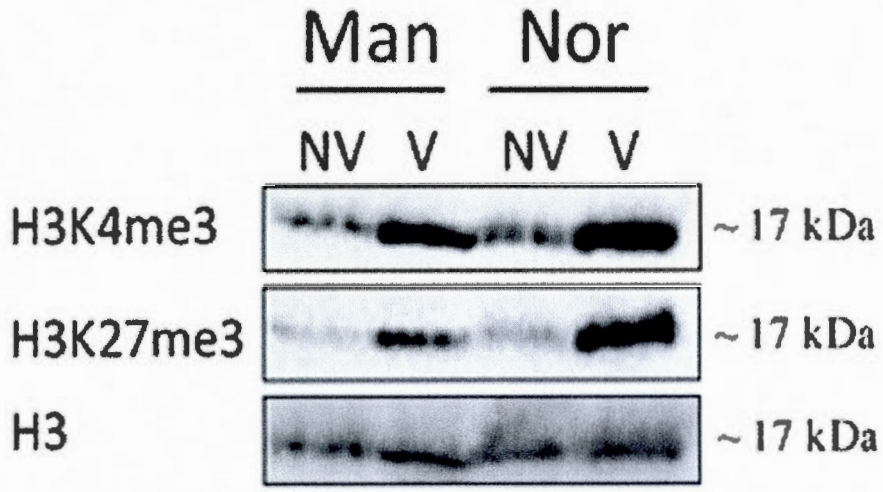

Figure 4.3 Immunoblot analysis of nuclear protein extracted from aerial plant tissues

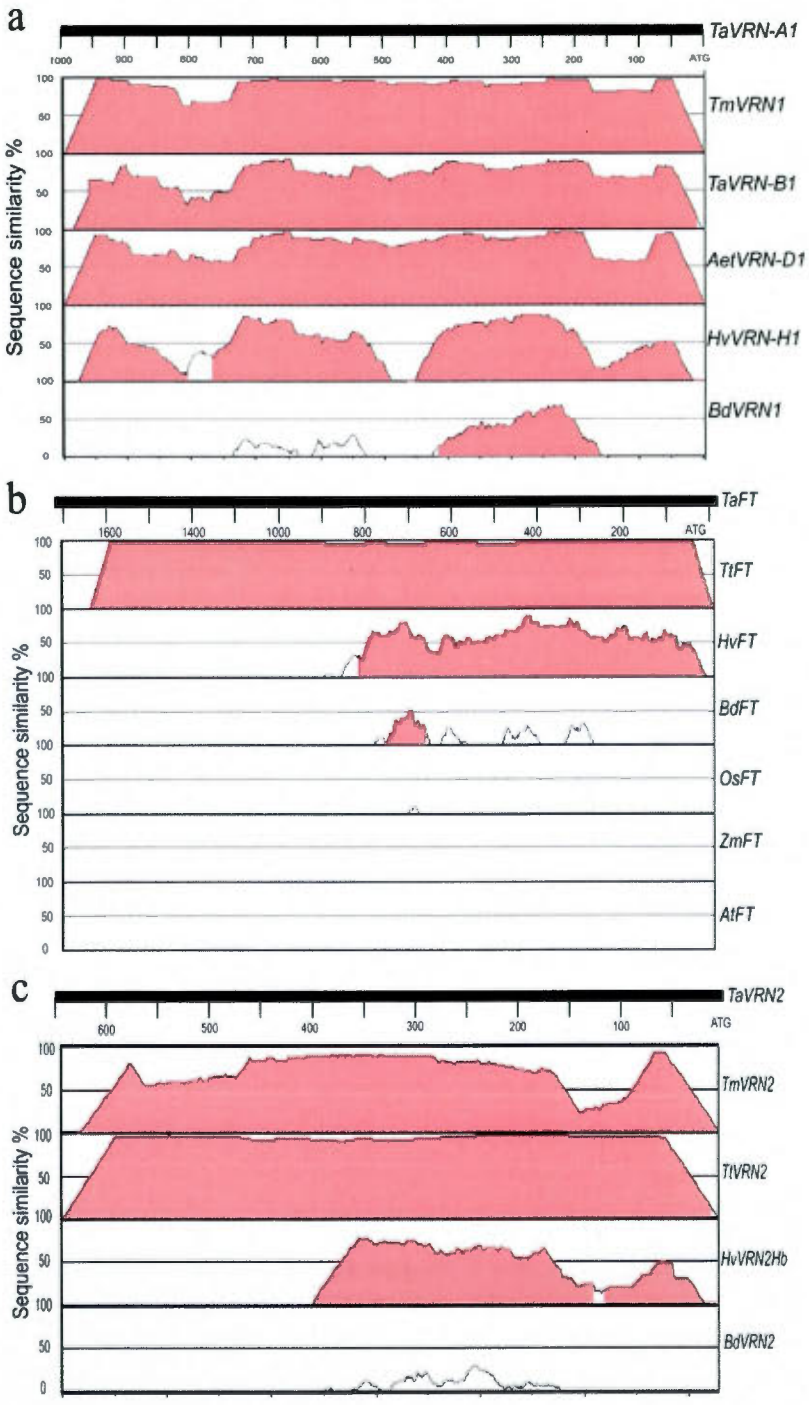

Figure 4.4 Pairwise alignment of *VRN1, FT and VRN2* promoter sequences from different species using mVISTA Shuffle-LAGAN program

Figure 4.5 Pairwise alignment of *VRN1* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2

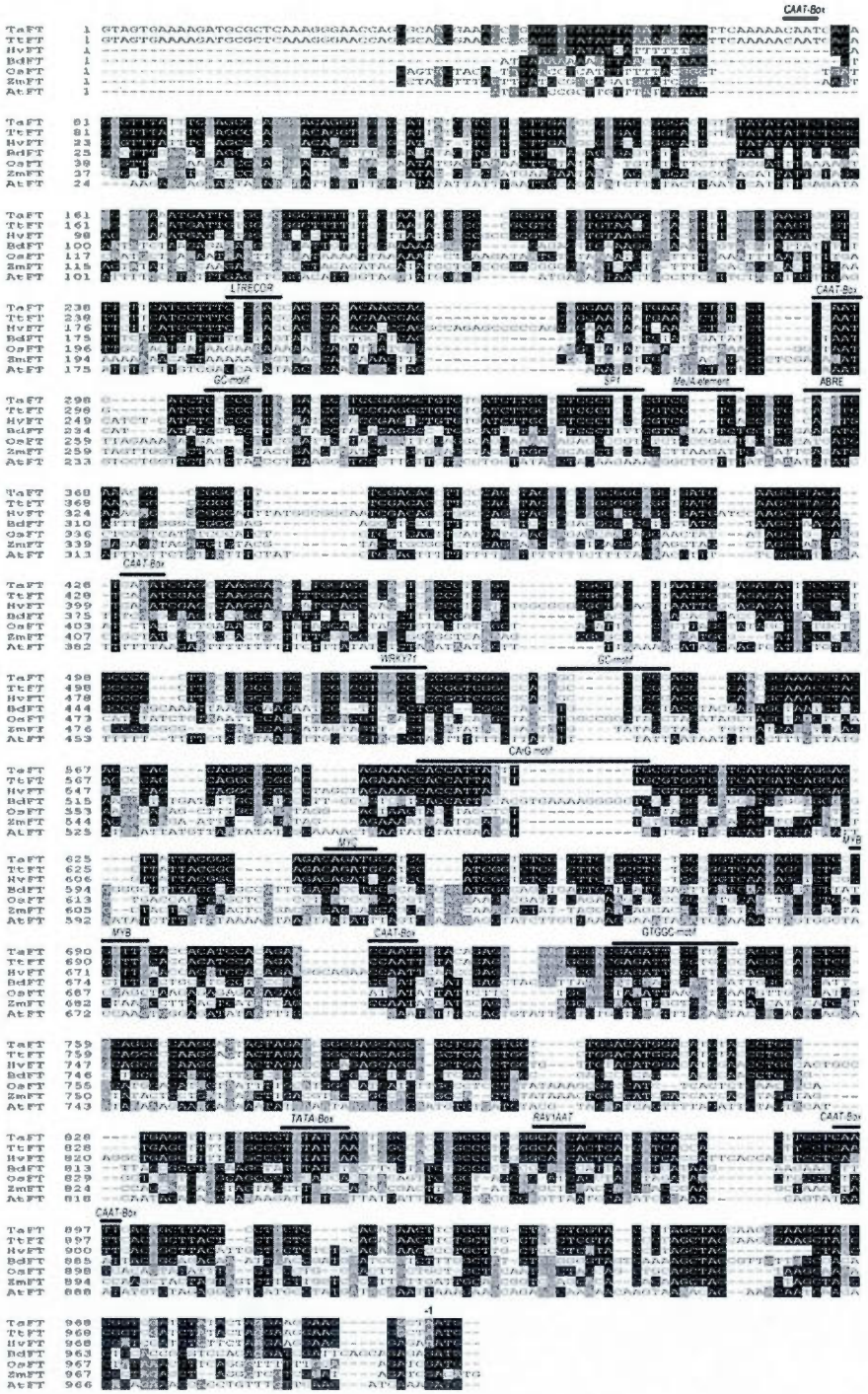

Figure 4.6 Pairwise alignment of *FT* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2

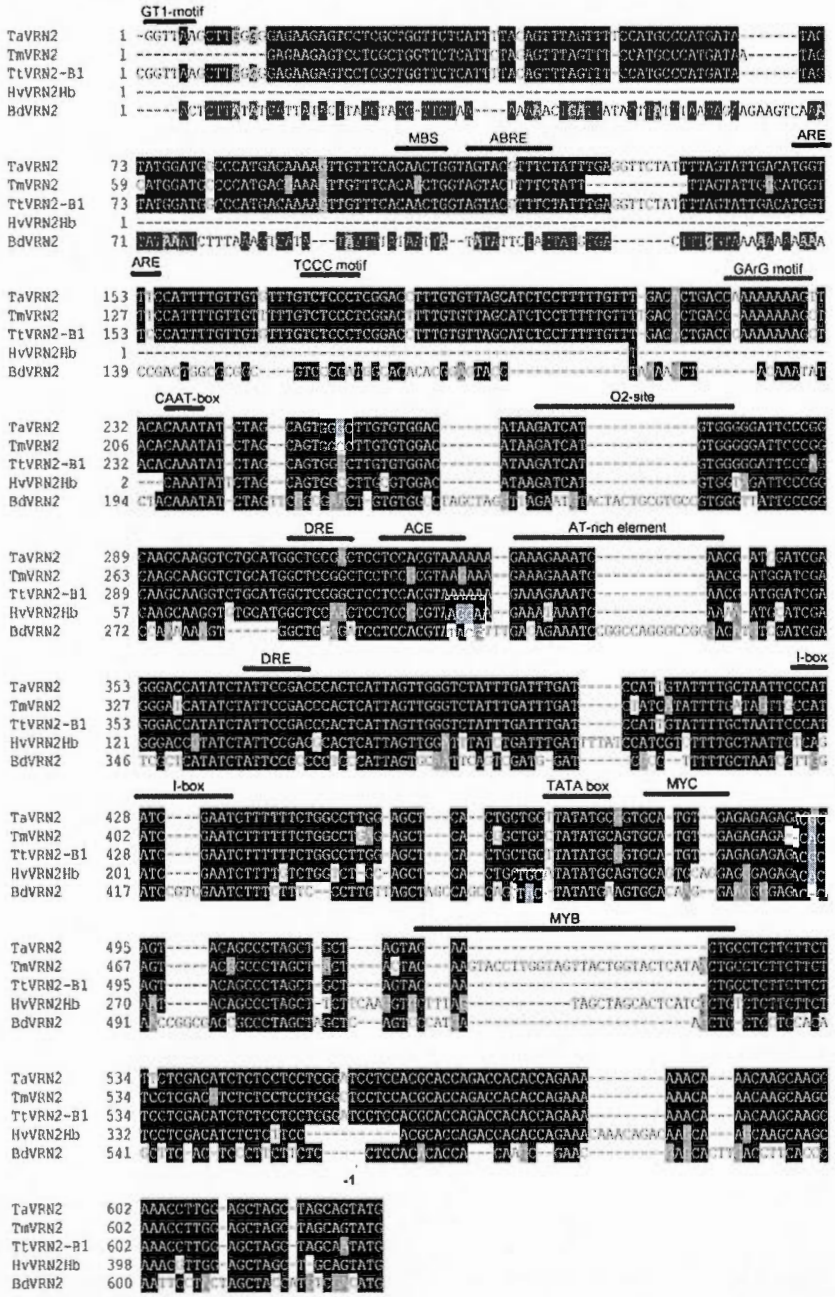

Figure 4.7 Pairwise alignment of *VRN2* promoter sequences showing *cis*-regulatory elements from different species using ClustalW2

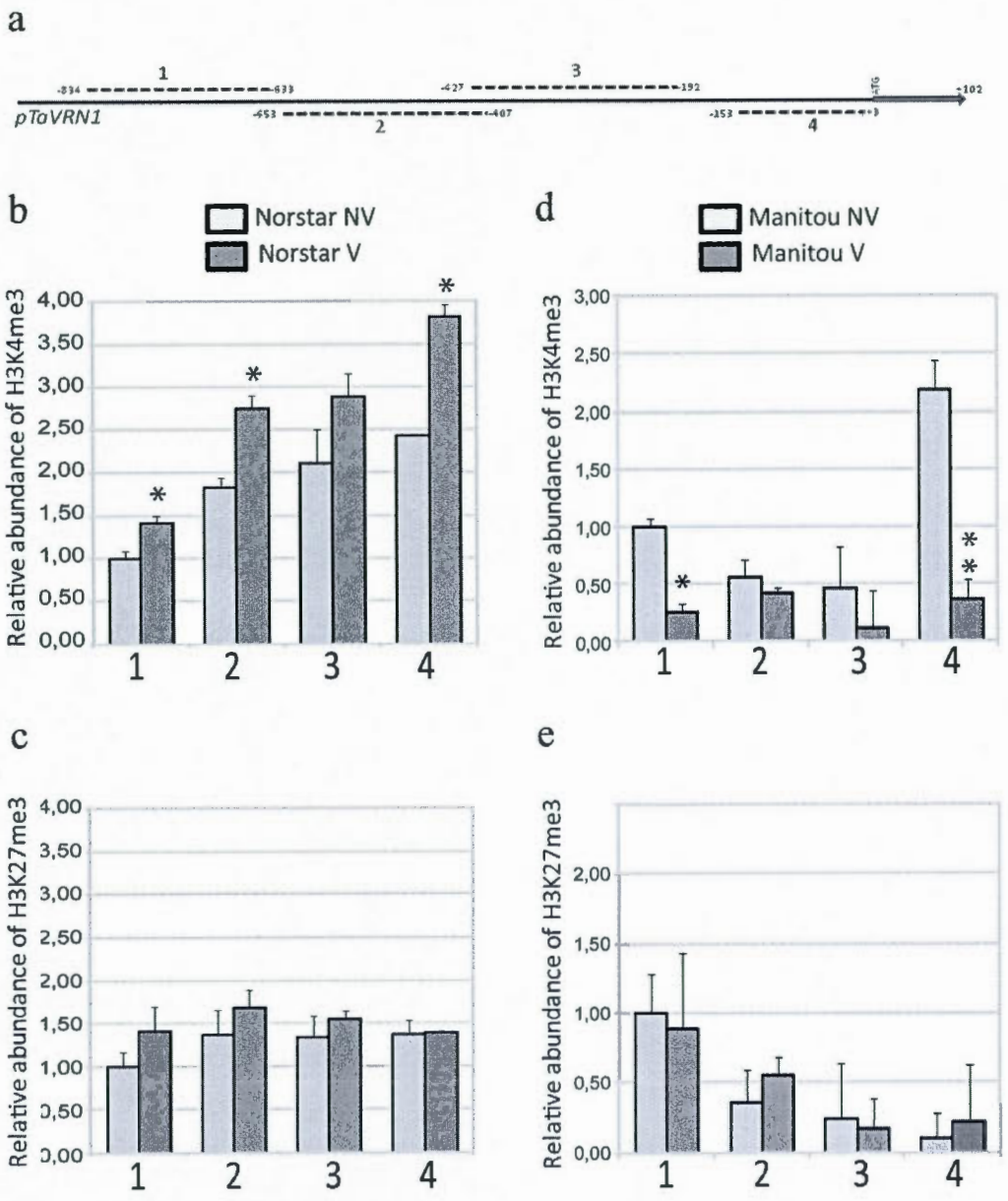

Figure 4.8 Effect of vernalization on histone modifications at different *TaVRN1* promoter regions in hexaploid wheat seedlings

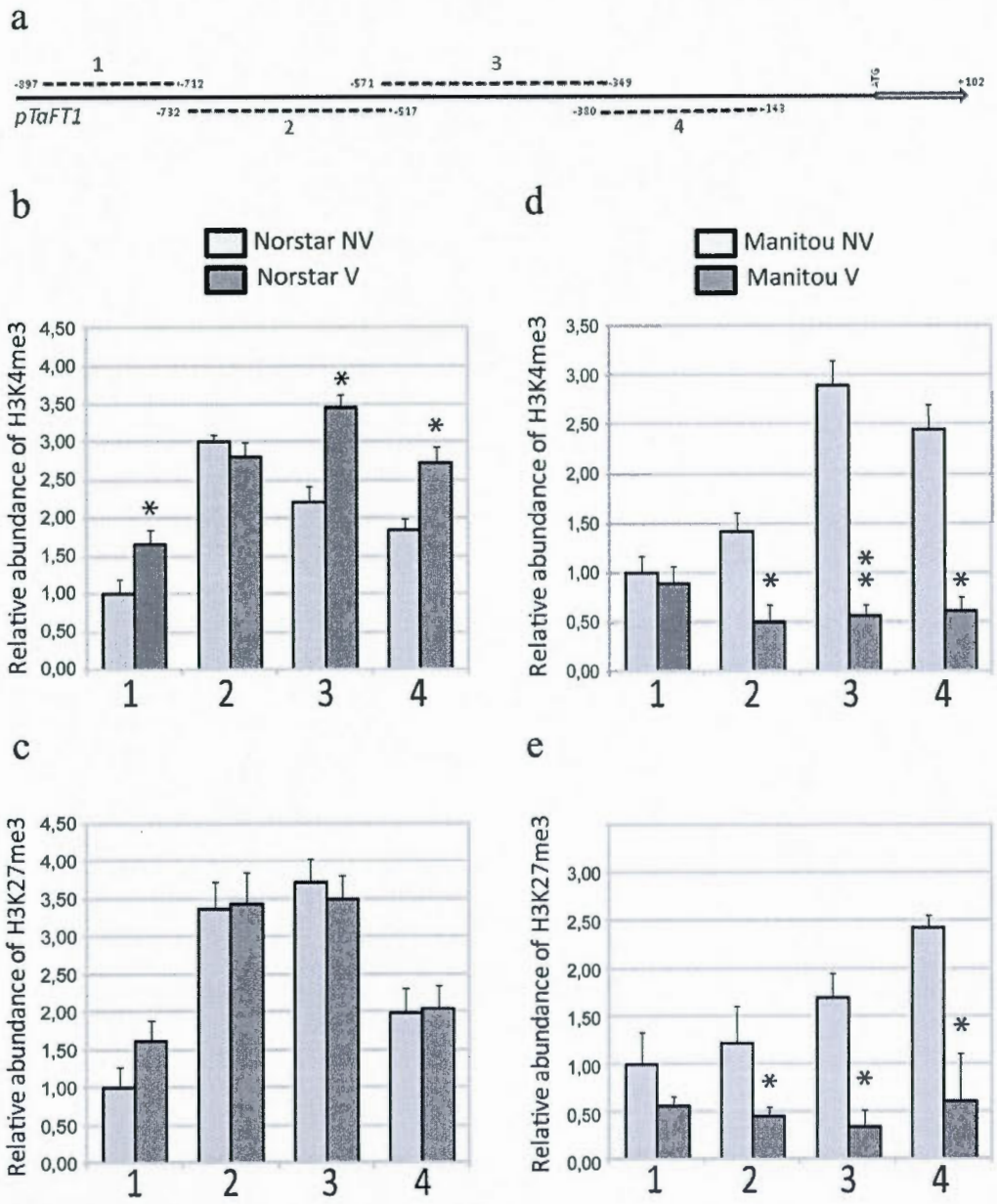

Figure 4.9 Effect of vernalization on histone modifications at different *TaFT1* promoter regions in hexaploid wheat seedlings

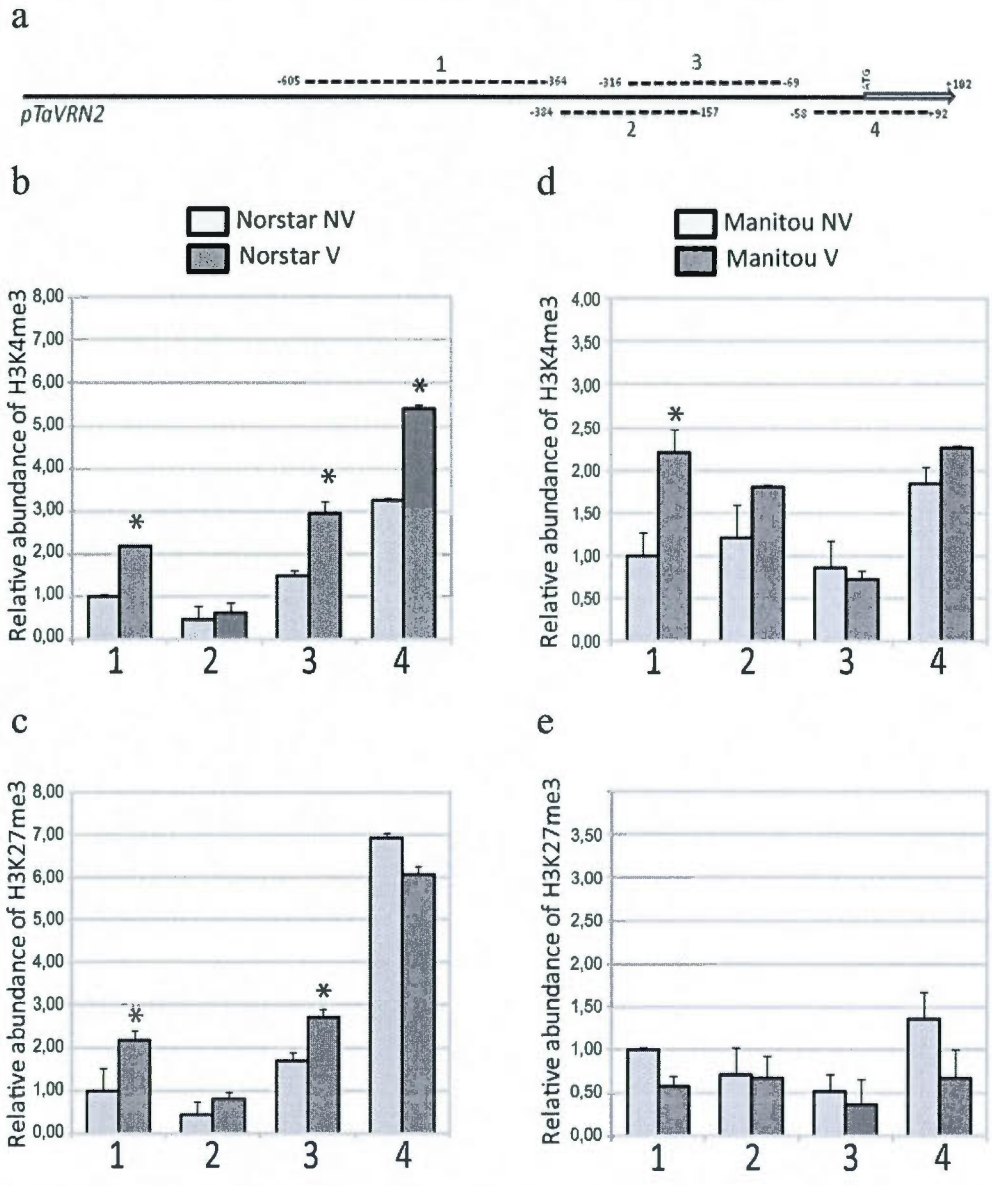

Figure 4.10 Effect of vernalization on histone modifications at different *TaVRN2* promoter regions in hexaploid wheat seedlings

>Promoter *TaVRN1_Cis*-elements analysis

CTGGTCGGTATACACGCACAGCACAGTACCCCTACTCCTAGGACTGGCGAGTATCTTTCATTCATTCCAGAAATA
CGCGGGTCGGCCAAAAGTAGAAAAATACACTGCGCCCACTCGATCCACGCAGCGCACTGCACTGCACTGCACAGC
AACGCTTCATGTCAAAAGTCGAGCTCAATCATGCACGCGATGGACGCGGCGCGAACGACCCGGGCGGCCCGCCGC
GCCCGCCCGCCCGCCCCGCAGCCGACCTCTCCCAAACGGGGCAAGCGAGACGGCCCAAAACGAGCAAGGAAAGCA
GCCTCCTACTGTGGCAGCCCGCCCCCACGACCGTCATCTAGCCTTCCATTCCATTTTCCCTGGACGGACCAGACC
CGTCCCGAGCCGCCCTGACCTAGCCAGCCAGCATTTCCTCTTTCGTCCCCCGCCGCCGTGACCAAAAAAGCAAAA
AAGGAAAAGGGGGAAATGCTAAAGGAAAAAACTCCGCTCTTTCCCTTCTTCTAGGCCTAGGGTACAGTAGAATAT
TATGAAAGGAAAAATTCTGCTCGTTTTTTTGCTCTGTGGTGTGTTTGTGGCGAGAGAAAATGATTTGGGGAAA
GCAAAATCCGGAGATTCGCACGTACGATCGTTCGACACGTCGACGCCCGGCGGGCCCGGGGTGGGGCATCGTGTG
GCTGCAGGACCGCGGGGCCCCGCAAAGCGGGCCGGGCCAATGGGTGCTCGACAGCGGCTATGCTCCAGACCAGCC
CGGTATTGCATACCGCGCTCGGGGCCAGATCCCTTTAAAAACCCCTCCCCCCCTGCCGGAATCCTCGTTTTGGCC
TGGCCATCCTCCCTCTCCTCCCCTCTCTTCCACCTCACGTCCTCACCCAACCACCTGATAGCCATGGCTCCGCCG
CCTCGCCTCCGCCTGCGCCAGTCGGAGTAGCCGTCGCGGTCTGCCGGTGTTGGAGGGTAGGGGCGTAGGGTTGGC
CCGGTTCTCGAGCGGAGATG

>Promoter *TaFT_Cis*-elements analysis

AAGAATCCAAAGGAAAAGATATAGGGTAGTGAAAAGATGCGCTCAAAGGGAACCAGGGCAACGAAAGCGGAGGGT
ATATTAAAAAGAAATTCAAAAACAATCAGAGGGTTTATTTCGAGCCCAGGGACAGGTGGAGCATTATCATCGTCC
TTGAGCCGGGACCGGCACTGTATATATATTCTCGCGGGAAAATGATTGTGGGGGGCTTTTTTCCTAATACGGCCC
GCGTCCCGTGTAAGCTAACGTTTCGGGAAAGTCCGTCTTCTTCATCCTTTCCGACCACGCACACAACCAGCAGCA
AGAATGAAACCTGTCCTCCAATGATCTCCCCCGTACGACCCATCCGAGGCTGTGTGATCTTGCTCTCCCTCCCCG
TCGTCACCATGCACGTGAAACGGCCGGGCTTTCGACACCTTCCCAGCTACGGCCGGCGGCAGCTGATGAAGCTTA
CATCAGATCGAGCCAAGGAAGCCTGCACCCCAGTCACCGTCGGCCGCTAGCTAATTGGCAGACATTCCCTGTGCC
CGCTTGCCGGCCGGCCGCGGCGTGACCGCCGGTCGACTCAAAGCCCCGGACGCAACGCAAACCTACACCCCAGCA
GGCACGGACAGAAACACCATTAATTTGCGTGGTGATCATGATCAGAGCTTATTACGGCAGACAGATGCATCCATC
GGTCTCGCTTCTGCCTGTGGGGGTCAAAAGCGCTGCCGGTTGCACCACATCCACAGAACCAATTGTACAGAGGGA
GGCGACGAGATTCCGTGGCCACGCCAGCTCGGCAGCGCCAAGGAGTACTAGAGCGGCGAGCAGCGGCTGAACTGG
TCTGGACATGGACATGTACCCTGCGTGGGCTTTTCGGCCCTATATAAAGTGGCCACCGGCCGTGGGGCAACACTC
ATCATCACCACTTCCTCAATTCACAGCTTACTCCTGCTCCAGAGAACTTCTGCTTGCTGCCTCGTACCCTAGCTA
GCAAGGCAAGCTAGCCGGTC

>Promoter_TaVRN2_*Cis*-elements analysis

GTTAAGCTTGGGGGAGAAGAGTCCTCGCTGGTTCTCATTTTACAGTTTAGTTTCCATGCCCATGATATAGTATGG
ATGGCCCATGACAAAAGTTGTTTCACAACTGGTAGTACGTTTCTATTTGAGGTTCTATTTTAGTATTGACATGGT
TTCCATTTTGTTGTGTTTGTCTCCCTCGGACCTTTGTGTTAGCATCTCCTTTTTGTTTGACACTGACCAAAAAAA
AGTTACACAAATATCTAGCAGTGGGCTTGTGTGGACATAAGATCATGTGGGGGATTCCCGGCAAGCAAGGTCTGC
ATGGCTCCGACTCCTCCACGTAAAAAAGAAAGAAATCAACGATCGATCGAGGGACCATATCTATTCCGACCCACT
CATTAGTTGGGTCTATTTGATTTGATCCATTGTATTTTGCTAATTCCCATATCGAATCTTTTTTCTGGCCTTGGA
GCTCACTGCTGCTTATATGCGGTGCATGTGAGAGAGAGACGCAGTACAGCCCTAGCTGCTAGTACAACTGCCTCT
TCTTCTTTCTCGACATCTCTCCTCCTCGGATCCTCCACGCACCAGACCACACCAGAAAAAACAAACAAGCAAGCA
AACCTTGGAGCTAGCTAGCAGTATGTCCATGTCATGCGATTTGTGCGGCGCCAACAACTGCTCGCGCCTCATGGT
CTCGCCCATTCATCATCATCATCACCATCATCAGGAGCACCAGCTGCGTGAGCACCAGTTCTTCGCCCAAGGCAA
CCACCACCACC

Figure S4.1 *TaVRN1*, *TaFT* and *TaVRN2* promoter from cv Norstar analysis

Figure S1: *TaVRN1*, *TaFT* and *TaVRN2* promoter from cv Norstar analysis

Using the plant CARE and PLACE programs from the web, putative regulatory cis-elements from *TaVRN1* and *TaFT* promoter were identified and underlined. *TaVRN2* promoter cis-elements were previously reported (Diallo et al, 2010). The *Cis*-regulatory elements highlighted in promoter sequences of the three genes are suggested to be targeted by proteins involved in response to vernalization and proteins involved in chromatin state organization (details in Table SI).

Table S4.1 Functions of identified *cis*-regulatory elements and functional "units" (PRE/TRE) in the promoter of *TaVRN1*, *TaFT1* and *TaVRN2* genes

Promoter *TaVRN1 Cis*-elements	Functions and description
CAAT	common cis-acting element in promoter and enhancer regions (CAAT-box) (**2 times**)
AACGAC	auxin-responsive element
GGGCGG	light responsive element
CCGCCGCGC	abscisic acid responsive element (motif IIb)
GCCGAC	DRE element
CACGAC	cis-acting regulatory element involved in light responsiveness (G-box)
CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness (CGTCA-motif)
GTCAT	cis-acting regulatory element required for endosperm expression (Skn-1-motif)
CCGTCC	cis-acting regulatory element related to meristem specific activation (CCGTCC-box)
CCCCCG	enhancer-like element involved in anoxic specific inducibility (GC-motif)
CATCGTGTGGC	part of a light responsive element (GTGGC-motif)
TTTAAAAA	core promoter element around -30 of transcription start (TATA-box)
CCTCGTTTTGG	CArG motif
CACGTC	**2 times** (C-box), cis-acting regulatory element targeted by bZIP transcription factors (**Golovnina et al, 2010**).
ACGT	**3 times**_cis-acting regulatory element targeted by bHLH and GATA1 transcription factors (**Golovnina et al, 2010**).
GCCAT	**5 times** (PRE and TRE motifs) (**Ringrose et Paro, 2004**)
GAAAA	**2 times** (PRE and TRE motifs) (**Ringrose et Paro, 2004**)
Promoter *TaFT1 Cis*-elements	**Functions and description**
CAAT	common cis-acting element in promoter and enhancer regions (CAAT-box) (**4 times**)
CCCCCG	enhancer-like element involved in anoxic specific inducibility (GC-motif) (**2 times**)
CCTCCCCGTC	light responsive element (SP1)
CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness (CGTCA-motif)
CACGTG	cis-acting element involved in the abscisic acid responsiveness (ABRE or G-box)
ATTAAT	part of a conserved DNA module involved in light responsiveness (BOX 4)
GATTCCGTGGC	part of a light responsive element (GTGGC-motif)
CTATATAAAG	core promoter element around -30 of transcription start (TATA-box)
CATTAATTTG	CArG motif
CACGTG	(G-box), cis-acting regulatory element targeted by bZIP transcription factors (**Golovnina et al, 2010**).
ACGT	**2 times**, cis-acting regulatory element targeted by bHLH and GATA1 transcription factors (**Golovnina et al, 2010**).
GAAAA	**3times**, (PRE and TRE motifs) (**Ringrose et Paro, 2004**)
Promoter *TaVRN2 Cis*-elements	**Functions and description**

ACGT	**3 times**, cis-acting regulatory element targeted by bHLH and GATA1 transcription factors (**Golovnina et al, 2010**).
GAGAG	(PRE and TRE motifs) (**Ringrose et Paro, 2004**)

Table S4.2 Accession numbers of promoter sequences of species used for pair wise alignment in this study

Promoter name	Accession number
TmVRN1	AY188331
TaVRN-B1	AY616453
AetVRN-D1	AY616454
HvVRN-H1	EU331770
Bd1VRN1	Bd1
TtFT	DQ890164
HvFT	EU007827
BdFT	Lcl Bd1:47477933
OsFT	AP007223
ZmFT	EU241906
AtFT	GQ395498
TmVRN2	AY485970
TtVRN2-B1	FJ173819
HvVRN2Hb	AY485978
BdVRN2	Bd3

Table S4.3 Primers used for cloning the promoters of *TaVRN1* and *TaFT1*, for the qPCR after chromatin immuno precipitation and the qRT-PCR for gene expression of *TaVRN-A1* and *TaFT-A1*. Primers used for promoter cloning and the gene expression of *TaVRN2* are previously reported (Diallo et *al* 2010).

	Primers –FORWARD	Primers -REVERSE
Promoters Cloning		
TaVRN1	5'-AGGTACCCCGCAGCCGACCTCTCCCAA-3'	5'-AGGATCCCTCCGCTCGAGAACCGGGCCAA-3'
TaFT	5'-CTCGTCTCCAGCTAACGTTCGTTGTGAGGGGTGG-3'	5'-GTTCTAGATAGCCCTTCCTTCCTAGTATAGAT-3'
Promoters ChIP		
pTaVRN1_1	5'-ATGTCAAAAGTCGAGCTCAATCA-3'	5'-GAAAATGGAATGGAAGGCTAGAT-3'
pTaVRN1_2	5'-AGCCTTCCATTCCATTTTCC-3'	5'-TTCTCTCGCCACAAACACAC-3'
pTaVRN1_3	5'-GTGTGTTTGTGGCGAGAGAA-3'	5'-GGGGAGGGGTTTTTAAAGG-3'
pTaVRN1_4	5'-TCTCCTCCCCTCTCTTCCAC-3'	5'-CATCTCCGCTCGAGAACC-3'
pTaVRN2_1	5'-AGAGTCCTCGCTGGTTCTCA-3'	5'-CACACAAGCCCACTGCTAGA-3'
pTaVRN2_2	5'-TCTAGCAGTGGGCTTGTGTG-3'	5'-TAAGCAGCAGTGAGCTCCAA-3'
pTaVRN2_3	5'-CCGACTCCTCCACGTAAAAA-3'	5'-CGAGGAGGAGAGATGTCGAG-3'
pTaVRN2_4	5'-CACCAGACCACACCAGAAAA-3'	5'-TGCTCCTGATGATGGTGATG-3'
pTaFT_1	5'-GGGACAGGTGGAGCATTATC-3'	5'-TTCATTCTTGCTGCTGGTTG-3'
pTaFT_2	5'-CAACCAGCAGCAAGAATGAA-3'	5'-CACAGGGAATGTCTGCCAAT-3'
pTaFT_3	5'-ACATCAGATCGAGCCAAGGA-3'	5'-GATGCATCTGTCTGCCGTAA-3'
pTaFT_4	5'-ATCAGAGCTTATTACGGCAGACAG-3'	5'-CCACTTTATATAGGGCCGAAAAG-3'
Ta18S_RNA	5'-ATGCCTAGTAAGCGCGAGTCAT-3'	5'-ACGGGCGGTGTGTACAAAG-3'
qRT-PCR expression level		
TaVRN-A1	5'-GGAGAGGTCACTGCAGGAGGA-3'	5'-GCCGCTGGATGAATGCTG-3'
TaFT-A1	5'-CAGGCCGGTCGATCTATACTA-3'	5'-TCCTGTTCCCGAAGGTCA-3'

4.8 DETAILED CHROMATIN IMMUNO PRECIPITATION (CHIP) PROTOCOL

http://www.epigenomenoe.net/researchtools/protocol.php?protid=13#reagents#reagents

Day 1

Chromatin Crosslinking

1. Harvest 1.5g seedlings (aerial part) and place them into a 50ml Falcon tube;
2. Rinse seedlings twice with 40ml bidistilled water. Remove as much water as possible after second wash;
3. Add 37ml 1% formaldehyde solution. Gently submerge seedlings at the bottom of the tube by stuffing the tube with nylon mesh. Screw on cap and poke cap with needle holes. Put in exsiccator and draw vacuum for 10 minutes;
4. Release vacuum slowly and shake exsiccator slightly to remove air bubbles. Seedlings should appear translucent;
5. Add 2.5ml 2M glycine to quench crosslinking. Draw vacuum for 5 minutes;
6. Again, release vacuum slowly and shake exsiccator slightly to remove air bubbles;
7. Remove nylon mesh, decant supernatant and wash seedlings twice with 40ml of bidistilled water; After second wash, remove as much water as possible and put seedlings between two layers of kitchen paper. Roll up paper layers carefully to remove as much liquid as possible.

At this step, plant material can be shock-frozen in liquid nitrogen and stored at -80°C. In my hands, this step provides the only possibility, where the protocol can be interrupted and still gives reproducible results.

Chromatin preparation

1. Precool mortar with liquid nitrogen. Add 2 small spoons of silicon dioxide (Sigma, S9887) and plant material. Grind plant material to a fine powder;
2. Use cooled spoon to add powder to 30ml of Extraction Buffer 1 (see buffers for ChIP protocol below) stored on ice. Vortex to mix and keep at 4°C until solution is homogenous;
3. Filter extract through Miracloth into a new, ice-cold 50ml Falcon tube. Rigidly press out solid material;

4. Repeat step 3;
5. Centrifuge extract using the Beckman JS 7.5 rotor (or equivalent) at 4000 rpm for 20 minutes at 4°C;
6. Gently pour off supernatant and resuspend pellet in 1ml of Extraction Buffer 2 by pipetting up and down. Transfer solution to Eppendorf tube;
7. Spin in cooled benchtop centrifuge at 13000 rpm for 10 minutes;
8. Remove supernatant and resuspend pellet in 300µl of Extraction Buffer 2 by pipetting up and down;
9. Add 300µl of Extraction Buffer 3 to fresh Eppendorf tube; Use pipette to carefully layer solution from step 8 onto it.
10. Spin in cooled benchtop centrifuge at 13000 rpm for 1 hour. In meantime, prepare 10ml Nuclei Lysis Buffer and 20ml CHIP Dilution Buffer. Put buffers in coldroom;
11. Remove supernatant and resuspend pellet in 500µl of cold Nuclei Lysis Buffer. Resuspend by pipetting up and down and by vortexing (see comment 1). Remove 10µl to run on an agarose gel (see comment 2);
12. Sonicate 4x 10 seconds, 40% duty cycle and 20% power (Bandelin Sonoplus HD 2070 with MS 73 probe). Put on ice for 1 minute between sonication steps (see comment 3 and comment 4);
13. Spin in cooled benchtop centrifuge at 13000 rpm for 10 minutes. Add supernatant to new Eppendorf tube;
14. Repeat step 13. Remove 10µl to run on an agarose gel;
15. Separate aliquots from steps 11 and 14 on 1.5% agarose gel. In the sonicated samples, DNA should be shifted and more intense compared to untreated samples and range between 200-2000bp, centering around 500bp.

Pre-clearing and immuno precipitation (IP)

1. Transfer two 150µl aliquots of chromatin solutions to separate Eppendorf tubes. Add 1350µl of CHIP Dilution Buffer per tube;
2. Prepare Protein A agarose beads pre-absorbed with sheared salmon sperm DNA (Upstate, Cat. 16-157) by rinsing 40µL 3 times with 1ml CHIP Dilution Buffer in an Eppendorf tube. Spin in cooled benchtop centrifuge for 30 seconds at 13000rpm between the washes to pellet the beads;
3. Add 1.5ml diluted chromatin solution from step 1 per 40µl of equilibrated beads. Rotate for 1 hour at 4°C (see comment 5);
4. Spin in cooled benchtop centrifuge for 30 seconds at 13000 to pellet the beads. Combine chromatin of identical genotypes in 14 ml Falcon tube (e.g. 3ml in this example, see step 1). Be careful not to contaminate solution with carry over of beads;
5. Store a 60µl aliquot of pooled chromatin at -20°C. This will serve as input control (see comment 6);

6. Add 600µl of pooled chromatin solution per IP to an Eppendorf tube with an appropriate antibody. We use ~10µg of antibody per IP. Also set up 600µl of chromatin solution without antibody, which serves as mock IP. Rotate overnight at 4°C.

Day 2

Collection, washes and elution of immune complexes

1. Prepare fresh CHIP Dilution Buffer and store it at 4°C;
2. Prepare Protein A agarose beads pre-absorbed with sheared salmon sperm DNA (Upstate, Cat. 16-157) by rinsing 40µl 3 times with 1ml CHIP Dilution Buffer in an Eppendorf tube. Prepare one aliquot of beads per IP. Spin in benchtop centrifuge for 30 seconds at 13000rpm between the washes to pellet the beads;
3. Add IPs and rotate for 1 hour at 4°C. In the meantime, prepare Elution Buffer and place it at 65°C (see comment 7);
4. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant (see comment 8);
5. Add 1ml of Low Salt Wash Buffer per tube. Rotate for 5 minutes at 4°C;
6. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant;
7. Add 1ml of High Salt Wash Buffer per tube. Rotate for 5 minutes at 4°C;
8. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant;
9. Add 1ml of LiCl Wash Buffer per tube. Rotate for 5 minutes at 4°C;
10. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant;
11. Add 1ml of TE Buffer per tube. Rotate for 5 minutes at 4°C;
12. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant;
13. Repeat TE wash. Spin in cooled benchtop centrifuge at 5000 rpm for 30 seconds to collect beads and discard supernatant;
14. Elute immune complexes by adding 250µl of Elution Buffer. Vortex briefly to mix and incubate at 65°C for 15 minutes. Spin in benchtop centrifuge at 13000 rpm for 30 seconds and transfer supernatant to a fresh Eppendorf tube;
15. Repeat elution and finally combine the two elutes;
16. Add 460µl of Elution Buffer to the 60µl input control aliquoted on Day 1 (comment 9).

Reverse crosslinking

1. Add 20µl of 5M NaCl to samples. Incubate overnight at 65°C.

Day 3

4.8.1.1 DNA cleanup

1. Add 10µl of 0.5M EDTA, 20µl 1M Tris-HCl pH6.5 and 1µl of 20mg/ml proteinase K per tube. Incubate for 1 hour at 45°C (see comment 10);
2. Extract samples with 1 volume of phenol-chloroform. Spin in cooled benchtop centrifuge at 13000 rpm for 15 minutes and transfer supernatant to 2ml reaction tube (see comment 11);
3. Precipitate DNA with 1/10 volume 3M NaOAc pH5.2 and 3 volumes absolute ethanol. Add 4µl glycogen (Roche, Cat. 901393) per precipitation. Incubate at -20°C for at least 1 hour;
4. Spin in cooled benchtop centrifuge at 13000 rpm for 15 minutes. Wash pellet with 1ml 70% ethanol and spin again. Discard supernatant and vacuum-dry pellet for 10 minutes. Dissolve DNA in 50µl 10mM Tris-HCl pH7.5. Proceed to PCR reactions.

Comments

Reviewed by: Olivier Mathieu, Paszkowski lab, Genève

1. We preclear each chromatin sample (in 15ml Falcon tubes, see comment #2) by adding 70µl of Protein A Agarose beads. Rotate 1 hour at 4°C.
2. We resuspend in 300µl of Nuclei Lysis Buffer by pipetting up and down and vortexing (keep solution cold between vortexing). Incubate for 20 minutes on ice.
3. Following this step, the chromatin solution can be frozen at -20°C.
4. We dilute 10 times with CHIP Dilution Buffer in 15ml Falcon tubes (add 2.7ml of CHIP Dilution Buffer). The point here is to dilute the 1% SDS to 0.1% SDS.
5. The solution should not foam during sonication. Cool the tubes with a mix of 100% ethanol in ice during the sonication step.
6. Supernatant of the "mock" sample can also be used as the input control (see comment #8).
7. Collect immune complexes with Protein A Agarose beads for 3 hours at 4°C.
8. Save 500µl supernatant of the "mock" sample. This will serve as the input control.
9. Do not add Elution Buffer to the Input control saved at comment #8.

10. We incubate for 3 hours at 45°C, with shaking.
11. After proteinase K treatment, the immunoprecipitated DNA can be purified using a silica-gel membrane (e.g. Qiagen PCR purification kit), eluated in 50µl and directly analyzed by PCR.

Buffers and products for ChIP protocol

Extraction buffer 1	**FOR 10 mL**	**ChIP dilution buffer**	**FOR 10 mL**
0.4M sucrose	2 mL 2M	0.01% SDS	10 µL 10%
10mM Tris-HCl pH 8	0.1 mL 1M	1.1% Triton X- 100	550 µL 20%
10mM MgCl	0.1mL 1M	1.2mM EDTA	24 µL 0.5M
5mM BME	3.5 µL 14.3M	16.7mM Tris-HCl, pH 8	167 µL 1.0M
CMP Inhibitor Cocktail	1 Tablet	167mM NaCl	334 µL 5.0M
Nano pure H_20	**Complete to 10 mL**	Nano pure H_20	**Complete to 10 mL**
Extraction buffer 2	**FOR 10 mL**	**Low salt wash buffer**	**FOR 10 mL**
0.25M sucrose	1.25 mL 2M	0.1% SDS	100 µL 10%
10mM Tris-HCl pH 8	100 µL 1M	1% Triton X-100	500 µL 20%
10mM $MgCl_2$	100 µL 1M	2mM EDTA	40 µL 0.5M
1% Triton X-100	0.5 mL 20%	20mM Tris-HCl, pH 8	200 µL 1.0M
5mM BME	3.5 µL 14.3M	150mM NaCl	300 µL 5.0M
CMP Inhibitor Cocktail	1 Tablet	Nano pure H_20	**Complete to 10 mL**
Nano pure H_20	**Complete to 10 mL**		
Extraction buffer 3	**FOR 10 mL**	**High salt wash buffer**	**FOR 10 mL**
1.7M sucrose	8.5 mL 2M	0.1% SDS	100 µL 10%
10mM Tris-HCl pH 8	100 µL 1M	1% Triton X-100	500 µL 20%
0.15% Triton X-100	75 µL 20%	2mM EDTA	40 µL 0.5M
2mM $MgCl_2$	20 µL 1M	20mM Tris-HCl, pH 8	200 µL 1.0M
5mM BME	3.5 µL 14.3M	500mM NaCl	1 mL 5.0M
CMP Inhibitor Cocktail	1 Tablet	Nano pure H_20	**Complete to 10 mL**
Nano pure H_20	**Complete to 10 mL**		
Nuclei Lysis Buffer	**FOR 10 mL**	**LiCl wash buffer**	**FOR 10 mL**
50mM Tris-HCl pH 8	0.50 mL 1M	0.25M LiCl	2.5 mL 1.0M
10mM EDTA	200 µL 0.5M	1% IGEPAL CA630	100 µL
1% SDS	0.500 mL 20%	1% deoxycholic acid Na	1.0 mL 1.0M
CMP Inhibitor Cocktail	1 Tablet	1mM EDTA	20 µL 0.5M
Nano pure H_20	**Complete to 10 mL**	10mM Tris, pH 8	100 µL 1.0M
		Nano pure H_20	**Complete to 10 mL**
Elution buffer	**FOR 10 mL**	**TE buffer**	**FOR 10 mL**
1% SDS	1 mL SDS 10%	10mM Tris-HCl, pH 8.0	100 µL 1.0M

0.1 M NaHCO3	1 mL 1 M NaHCO3	1mM EDTA	20 µL 0.5M
Nano pure H_20	**Complete to 10 mL**	Nano pure H_20	**Complete to 10 mL**

Products

Protein A Agarose Beads (SIGMA Cat P3476 or Upstate Cat 16-157)
Yeast tRNA (Invitrogen Cat 15401-029)
BSA Purified (Bio Labs 10 mg/mL)
RNAse A (Qiagen 100 mg/mL)
Antibodies K9 or K4 modifications.
Proteinase K (Analytical Biochemical): 10 mg/ml, dissolve in dH_2O, store at –20C, effective concentration 50 ug/ml, 37-56 C.
Chromatin Immunoprecipitation (CHIP) Assay kit: Upstate Cat. No. 17-295 (209$), store at 4C for 1 year. For 22 chip in 2 ml reaction, or 44 chip in 1 ml, supplemented with own-made buffers.
Glycogen (Roche Cat 901393 or MBI)

CHAPITRE V

TRANSCRIPTOME ANALYSIS OF *MVP* MUTANT REVEALS IMPORTANT CHANGES IN GLOBAL GENE EXPRESSION AND A ROLE OF METHYL-JASMONATE IN FLOWERING

Transcriptome analysis of *mvp* mutant reveals important changes in global gene expression and a role of methyl-jasmonate in flowering

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Description du rôle de chacun des auteurs en commençant par l'auteur principal : tâches et responsabilité spécifiques, nature de la contribution à l'article (rédaction, expérimentation, figures, recherche bibliographique, etc.)

AOD, ZA et FS ont pensé et conçu les expériences. AOD, ZA, MB, MAB-A et AM ont effectué les expériences. AOD a procédé à la sélection des échantillons pour l'expérience Microarray et pour la mesure du contenu en jasmonates, a effectué l'analyse des données Microarray, la confection des figures et la recherche bibliographique avec ZA et FS. AOD MH et FS ont écrit le manuscrit. ZA a conduit et supervisé l'expérience de traitement au methyl-jasmonate et MB, MAB-A et AOD ont participé à l'expérience de traitement au methyl-jasmonate. ZA, MB et MAB-A ont effectué l'analyse de plantes pour mesurer le retard de floraison. ZA a effectuée une lecture critique du manuscrit. MAB-A a effectué l'analyse des données qPCR avec AOD et FS. AM a effectué la mesure du contenu en jasmonates.

ATTESTATION DE L'AUTEUR PRINCIPAL ET DU DIRECTEUR DE RECHERCHE

L'auteur principal de l'article intitulé: **Transcriptome analysis of *myp* mutant, lacking vernalization gene *VRN1*, reveals important changes in global gene expression and a role of methyl-jasmonate in flowering**

soumis pour publication ☐, accepté pour publication ☐, ou publié ☐ dans ____ en préparation pour publication ____

Auteur principal	Nom Amadou Oury Diallo	Signature Amadou Oury Diallo	Date 6/09/11
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5.1 RÉSUMÉ

Des plantes d'un mutant *mvp-1* (maintien de la phase végétative) du blé *Triticum monococcum* L., précédemment identifié comme ayant des délétions qui incluent les gènes *AGLG1*, *CYS*, *PHYC*, *VRN1* et possiblement d'autres, sont incapables de fleurir. Pour déterminer l'impact de ces délétions sur le transcriptome du blé, des analyses de microarray du mutant *mvp* et du blé de type sauvage ont été effectuées. L'analyse indique que ces délétions dans les plantes du mutant *mvp* conduisent à la régulation différentielle de 368 gènes. Parmi les gènes hautement régulés, on compte ceux associés à la réponse aux pathogènes (PR) et aux jasmonates. Ces résultats suggèrent que ces délétions dans les plantes du mutant *mvp* sont associées à l'activation de la réponse moléculaire du mécanisme de défense de ces plantes modulée par la biosynthèse des jasmonates ce qui pourrait affecter leur floraison. Pour confirmer cette observation, nous avons mesuré la teneur en methyl jasmonate (MeJA) et en acide jasmonique (JA) dans les plantes homozygotes du mutant *mvp*, des plantes hétérozygotes et des plantes de type sauvage. Le contenu de JA était comparable dans tous les types de plantes tandis que le contenu en MeJA était six fois plus élevé dans les plantes du mutant *mvp-1* en comparaison du contenu du MeJA aux plantes de type sauvage et hétérozygotes. Un traitement avec 150 µM de MeJA sur du blé de printemps hexaploïde (cv Manitou) montre un retard de floraison de deux semaines et une croissance réduite des plantes. Ce retard dans la floraison a été associé à la répression significative des gènes *FLOWERING LOCUS T like 1* (*FT1*) et un gène putatif *Circumsporozoite* (*Cir*). L'effet du MeJA sur FT, démontre que cette hormone est un facteur important dans le contrôle de la floraison.

5.2 ABSTRACT

The *maintained vegetative phase 1*(*mvp-1*) mutant plants from *Triticum monococcum L.*, previously reported as having deletions that include the genes *AGLG1*, *CYS*, *PHYC, VRN1* and possibly others are incapable of flowering. To determine the impact of these deletions on wheat transcriptome, microarray analysis of *mvp* and wild type wheat were performed. The analysis indicates that deletions in the *mvp* mutant plants resulted in the differential regulation of 368 genes. Among the up-regulated genes, several belong to the pathogenesis related (PR) genes and jasmonates responsive and biosynthesis genes. This result raised the hypothesis that the deletions in the *mvp* mutant plants may have an impact on jasmonate biosynthesis and affect flowering. To confirm this hypothesis, we measured the methyl jasmonate (MeJA) and jasmonic acid (JA) content in *mvp* and wild type plants. The content of JA was comparable in all plants while the content of MeJA was six fold higher in *mvp-1* plants compared to the wild type plants. When spring hexaploid wheat (cv Manitou) was treated with 150 μM MeJA, we observed that flowering was delayed by two weeks was associated with significant repression of *FLOWERING LOCUS T like 1* (*FT1*). The effect of MeJA on FT, demonstrates that this hormone is an important factor implicated in flowering control.

5.3 INTRODUCTION

The transition from the vegetative to the reproductive phase is a critical event in the life cycle of seed-propagated plants. In *Arabidopsis*, four major pathways are implicated in flowering promotion: the vernalization, photoperiod, autonomous and gibberellin (GA) pathways (Boss *et al.*, 2004). Vernalization and photoperiod pathways are integrated by *FT* and its activity is directly suppressed by *FLC* (Searle *et al.*, 2006). FT functions as a flowering promoter and acts as a long-distance flowering signal (*florigen*). FT encodes a protein similar to the animal Raf kinase inhibitor-like protein (Kardailsky *et al.*, 1999 ; Kobayashi *et al.*, 1999). Expression of *CO* peaks during the light period under long day (LD) conditions, resulting in early flowering by activating *FT* expression (Yanovsky et Kay, 2003). The *PHYC* gene also contributes to variation in flowering time (Balasubramanian *et al.*, 2006), and the *PHYA* and *PHYB* are known to be involved in the posttranscriptional regulation of CO and therefore, contribute to the regulation of the photoperiod pathway in *Arabidopsis* (Valverde *et al.*, 2004). In rice, *Heading date 3a* (Hd3a) was detected first as a quantitative trait locus (QTL), which promotes flowering under short-day (SD) conditions. *Hd3a* encodes an ortholog of *Arabidopsis* FT. Hd3a/FT proteins are about 22 kDa in size, and their overall structures are similar to mammalian phosphatidyl ethanolamine-binding proteins or Raf-kinase inhibitor proteins (RKIP) (Ahn *et al.*, 2006). Recently it has been shown that Hd3a/FT interacts with 14-3-3 proteins in the apical cells of shoots, yielding a complex that translocates to the nucleus and binds to the *Oryza sativa* (Os)FD1 transcription factor, a rice homologue of *Arabidopsis thaliana* FD. The resultant ternary 'florigen activation complex' (FAC) induces transcription of the homologue of *A. thaliana APETALA1* (*AP1*), the homologue of wheat *VRN1*, or rice *OsMADS15* which leads to flowering (Taoka *et al.*, 2011).

It wheat, a mechanism was proposed where TaFT1 interacts with TaFDL2, which has the ability to bind the wheat *TmVRN1* promoter (Li et Dubcovsky, 2008). Taken together these findings indicate that FT1 may interacts with 14-3-3 proteins in wheat. *FT1* integrates the signals from the photoperiod pathway through its interactions with *PPD1* and *CO* in temperate cereals (Turner *et al.*, 2005). Most of the natural variation in the response to photoperiod is concentrated in the *PPD1* gene (Beales *et al.*, 2007 ; Turner *et al.*, 2005 ; Wilhelm, Turner et Laurie, 2009) whereas variations in the response to vernalization occurs in the *VRN1*, *VRN2* and *VRN3* genes (Beales *et al.*, 2007 ; Distelfeld, Li et Dubcovsky, 2009 ; Trevaskis *et al.*, 2007). The timing of floral transition is associated with the heading time in cereal crops such as wheat and barley, and constitutes an important character because of its influence on adaptability to various environmental conditions. Bread wheat (*Triticum aestivum*, 2n = 6x = 42, genome constitution *AA BB DD*) is grown in a wide range of environmental conditions and its wide adaptability results from the variation in heading time among cultivars. The bread wheat genome originated from three diploid ancestral species: the A genome came from *Triticum urartu*, the B genome came from *Aegilops speltoides* and the D genome came from *Aegilops tauschii* (Feldman, Bonjean et Angus, 2001).

The genetic control of heading time in wheat is clarified by many genetic studies and three characteristics have been identified: vernalization requirement, photoperiod sensitivity and narrow-sense earliness (Worland *et al.*, 2001). Plants that require vernalization accelerate spike primordium formation after a long exposure to cold temperatures while the vernalization insensitivite species (spring habit) does not require cold treatment to accelerate flowering. Vernalization is regulated by the vernalization genes, *Vrn-A1*, *Vrn-B1* and *Vrn-D1* located respectively on chromosomes 5A, 5B and 5D of bread wheat (Worland *et al.*, 2001). *Vrn-A1*, *Vrn-B1* and *Vrn-D1* are three homoeologs of the *Vrn-1* gene. Using a map-based method, *Vrn-A^{m}1* was isolated, in diploid einkorn wheat *Triticum monococcum* (2n = 2x = 14,

A^mA^m), the ortholog of *Vrn-A1* and this gene was named *VRN1* (Yan *et al.*, 2003). In hexaploid wheat, it has been identified as *TaVRT-1* (*Triticum aestivum* Vegetative to Reproductive Transition-1), (Danyluk *et al.*, 2003) or *WAP1* (wheat *AP1*) (Murai *et al.*, 2003). *VRN1* is a flowering promoter that has an indispensable role in the floral transition pathway of wheat based on transgenic and mutant analyses (Loukoianov *et al.*, 2005 ; Murai *et al.*, 2003 ; Shitsukawa *et al.*, 2007).

The identification of a non-flowering cultivated diploid einkorn wheat mutant (*Triticum monococcum* ssp. *monococcum* L., $2n = 2x = 14$, genome A^mA^m) lacking *VRN1* gene, provided an important tool to understand the function and regulation of VRN1. This mutant was induced by an ion-beam treatment that shows a *maintained vegetative phase* (*mvp*) phenotype and does not transit from the vegetative to the reproductive phase. It was recently shown that the region deleted covers more than the *VRN1* gene and its promoter (Distelfeld et Dubcovsky, 2010) and may affect aspects of plant development others than flowering. Among the deleted genes in the *mvp* mutant plants, the *CYSTEINE PROTEINASE* gene (*TmCYS*) and *PHYTOCHROME C* gene (*TmPHYC*) were identified (Distelfeld et Dubcovsky, 2010). The first gene encodes a protein that belongs to a family of proteins involved in variety of proteolytic functions in plants, particularly those associated with the processing and degradation of seed storage proteins and fruit ripening. These proteins are also induced in response to stresses such as wounding, cold, drought and in programmed cell death and senescence processes (Prins *et al.*, 2008). The *TmPHYC* gene belongs to a family of red/far-red photoreceptors that includes the *PHYA* and *PHYB* genes (Devos *et al.*, 2005 ; Furuya, 1993). The association of the *PHYC* gene with the regulation of flowering initiation and the known role of the phytochromes in light signalling suggests that the deletion of *TmPHYC* in the *mvp* mutant may have an effect on the regulation of the flowering promoter gene *TmFT1*. A putative role of the *PHYC* gene in the down-regulation of *FT1* expression is supported by the rapid response of these genes to light signals. When light conditions change from dark to

light or from light to dark, a rapid change in *FT1* transcript levels is observed and a current flowering model proposes an effect of photoperiod on the regulation of *FT1* (Shimada *et al.*, 2009 ; Trevaskis *et al.*, 2007).

To identify genes associated with the absence of *VRN1* and the other genes reported in *mvp* mutant, we analysed the transcriptome of both *mvp* mutant and the wild type plants using Affymetrix GeneChip wheat genome array. Results obtained were complemented by molecular and physiological analyses which provide more information of the impact of the deletions on gene expression and plant growth and development. This study revealed that the *mvp* mutant caused the up-regulation of PR genes related to the plant defense mechanism and jasmonates responsive genes and the accumulation of methyl jasmonate (MeJA) suggesting a possible effect of MeJA in flowering control. Treatment with MeJA caused a delay in flowering in *Triticum aestivum* wheat and repressed *TaFT1* supporting a role of MeJA in flowering control. This delay in flowering is also associated with the repression of *TaCir*, a novel gene that may be implicated in the flowering process in wheat.

5.4 MATERIALS AND METHODS

5.4.1 PLANT MATERIAL AND GROWTH CONDITIONS

Two wheat spring cultivars (*Triticum aestivum*, 2n x 6 = 42), Manitou and Bounty and Einkorn wheat (*Triticum monococcum*, 2n x 2 = 14, A^mA^m) were grown in a controlled growth chamber as previously described (Diallo *et al.*, 2010). Briefly, plants were grown in a growth chamber at 20°C under long day conditions (LD) (16 hours at 175 µmol m^{-2} s^{-1} and 8 hours dark) as specified for each experiment. The einkorn wheat mutant, *mvp* (Shitsukawa *et al.*, 2007) and the wild type of einkorn wheat (WT) as control were used for microarray and expression analyses. The *mvp* mutant was identified by the absence of *VRN1* expression and its inability to transit from the vegetative to reproductive phase. The hexaploid spring wheat cultivar (cv Manitou) is used to investigate the link between jasmonates and *VRN1* gene expression.

5.4.2 MUTANT PLANTS (*mvp*) IDENTIFICATION BY RT-PCR

Total RNA was extracted from the whole aerial part using TRIzol Reagent from Invitrogen and subsequent RT-PCR was used to identify homozygous (*mvp/ mvp*) or heterozygous (*Mvp / mvp*) *mvp* mutant plants after growth under long day conditions for the appropriate time of each experiment.

5.4.3 MICROARRAY DATA ANALYSES

Total RNA was isolated from the whole aerial part of 5 *mvp* mutant plants and from 3 wild type plants for each biological replicate using using TRIzol Reagent from Invitrogen. Three biological replicates were used for each type of plants. The RNA

quality was assessed on agarose gels and with the Bioanalyzer 2100 (Agilent). Microarray profiling was performed according to Affymetrix protocols at the Functional Genomics Platform of McGill University and Génome Québec Innovation Centre using the Affymetrix GeneChip® Wheat Genome Array. The microarray data were analyzed using FlexArray software (1.6.1) from Genome Quebec (GQ and McGill Univ. Innovation Centre) that contains the robust multiarray average (RMA) software used for data normalization. A two-fold cut-off value of expression and a P value ≤ 0.05 were set to indicate differential gene expression between *mvp* and wild type plants. Overall, this excludes 99.4 % of the genes on the microarray (60822 of 61,290) showing that most of the genes are expressed at a similar level between the two types of plants. Genes that are differentially expressed in *mvp* mutant compared to the wild type plants were selected and classified as regulated genes. These genes were subdivided in two groups: up-regulated (2-fold and up, 263 genes) and down-regulated genes (-2-folds and down, 105 genes). Each gene was subjected to BLAST search against Genbank and Uniprot databases to obtain their Genbank accession number, UniProt or NCBI description.

5.4.4 QUANTIFICATION OF JASMONATES

Whole aerial part of wheat plants were cut, and quickly grounded in dry ice. Then, 500 mg from powdered plants were extracted twice using 2 mL of MeOH /H_20/ HOAC (90:9:1 v/v/v) as described (Segarra *et al.*, 2006). The tubes were shaked on a vortex for 2 h at 4°C, and centrifuged at 4000 rpm for 5 min at 4° C. The supernatant was dried under gentle stream of N_2 and the dried powder reextracted by adding 250 µl of MeOH and then 250 µl of MeOH / H_2O (15:85). Samples were filtered on 0.4 µm syringe mini filters before being injected into HPLC / MS. Benzoic acid was used as internal standard according to Hou et al. (Hou *et al.*, 2008). LC-MS analyses were carried using an Agilent 1200 HPLC system with binary pump, in-line degasser, high performance auto-sampler and thermo-stated column division, using a linear gradient

of 20% to 95% methanol: 0.1% HCOOH /water for 20 min with a flow rate of 0.35 ml/min on an Agilent SB-C18 column (2.1 ×30mm; particle size, 3.5μm), and a column temperature of 25° C. The HPLC instrument was connected to an Agilent 6410 quadrupole (triple Q) mass spectrometer using electrospray ionisation in both negative and positive ESI mode for the analysis of jasmonic acid and its methyl ester, respectively, with the following conditions: capillary voltage: 3000 volts, nebulizer pressure: 60 PSI, gas temperature 300°C, drying gas: 5 L/min and a dwell time of 75 ms, and the data was processed using the Mass Hunter software. For MS/MS and MRM analyses, the collision energies (CE) were optimized; a (CE) of - 5eV and + 25 eV was applied as the optimum energy of collision for the analysis of jasmonic acid, and methyl jasmonate, respectively. The scan range was from 200 to 900 m/z. For jasmonic acid (JA), MRM acquisition was carried out by monitoring transitions of the combination of the parent ion mass in negative mode, 209 m/z and the fragment ion of highest abundance 59; whereas the transition was monitored in positive mode between 225 and 133 for methyl jasmonate (MeJA). References of JA and MeJA were used to compare the corresponding retention times and mass spectral profiles. An external calibration curve was constructed using different concentrations of jasmonic acid and methyl jasmonate for quantification purposes, along with the use of benzoic acid as internal standard. Three biological replicates were used and the analysis was repeated at least two times, with three injections for each sample.

5.4.5 EFFECTS OF METHYL JASMONATE (MeJA) ON FLOWERING TIME IN WHEAT

To determine the effect of MeJA on flowering time and gene expression, three week- old spring wheat (cv Manitou), were sprayed with 150 μM MeJA every day for two weeks. The phenological development of the plants was determined by measuring the final leaf number (FLN) as previously described (Wang *et al.*, 1995). The FLN on the main shoot of each plant at flag leaf stage was recorded. After the

end of hormonal treatment, the number of flowering plants was counted every day for two weeks. The percentage of plants with spikelets for a given week was calculated. After, the emergence of spikelets, the length of the main shoot was measured. This experiment was repeated six times.

5.4.6 FREEZING TOLERANCE AND COR PROTEINS EXPRESSION IN *mvp* AND WILD TYPE PLANTS

For low temperature treament, 3 week-old soil-grown plants were transferred to 4°C under the same long day photoperiod conditions for cold-acclimated (CA) plants. NA and CA soil grown plants were subjected to two separate freezing test experiments. We analysed 24 plants for heterozygous and 12 plants for wild type and homozygous *mvp* plants for each freezing test experiment. The freezing test is described in our previous report (Diallo *et al.*, 2010).

To test if the absence of VRN1 affects the ability of *mvp* plants to accumulate COR proteins, equal amounts of total proteins from the whole aerial part were used after extraction by grinding in a precooled motar with 0.1M TRIS-HCl, pH 8.5 containing 1 mM phenyl-methylsulfonyl fluoride (PMSF). The extract was centrifuged for 5 min at 12000 *g* at 4°C and the supernatant was adjusted to final buffer concentration with 2X SDS electrophoresis sample buffer (Laemmli, 1970). Samples were separated on a 12% SDS–polyacrylamide gel and transferred electrophoretically for 1 hour to a 0.45 µm polyvinylidene fluoride (PVDF) membrane (Life Sciences BioTrace™). The membrane was blocked in a 4% (w/v) solution of reconstituted skimmed milk powder prepared in PBS containing 0.2% (v/v) Tween 20 and then probed with different antibodies: anti-Wheat cold specific 120 (WCS 120) at 1: 20,000, anti-Wheat cold specific 19 (WCS 19) at 1: 10,000, anti-*Triticum aestivum* ice recystallization inhibition (TaIRI) at 1: 5,000 and anti-Wheat cold-regulated 410 (WCOR 410) at 1: 5,000 dilution overnight. After washing with

three times 20 min with PBS–Tween 20, the proteins recognized by the primary antibody were revealed with a goat anti-rabbit HRP conjugated IgG (Catalog # 12-348) at a dilution of 1: 20,000. The complexes were visualized using the enhanced chemiluminescent detection system using the HyGLO™ Chemiluminescent HRP Antibody Detection Reagent and HyBlot CL™ Autoradiography Film (Cat. No. E3018 DENVILLE Scientific Inc. P.O. Box 4588 Metuchen, NJ 08840-4588 USA, www.denvillescientific.com).

5.4.7 QUANTITATIVE REAL TIME PCR (qRT-PCR)

Three microgram of total RNA was reverse-transcribed (RT) and subjected to quantitative real-time PCR. RT-PCR analyses were performed using SuperScript™ First-Strand Synthesis System for RT-PCR according to instructions (Invitrogen). Transcript level of several genes (Table II) was measured by RT-PCR and qRT-PCR using Sybr Green. The program used for the PCR was: first step of activation at 95°C for 2 min, 35 cycles with 95°C for 30 sec, 58° C for 30 sec and 72° C for 1 min. The last step is an extension at 72° C for 10 min. Specificity of the RT-PCR analysis products was assessed by gel electrophoresis. A single product with the expected length was obtained for each reaction. Real-time PCR analysis was performed in the Light Cycler Roche 480 according to the manufacturer's protocol. The program used contains four steps. The first step is the initial activation at 95°C for 15 min followed by the PCR amplification performed up to 40 cycles with 95°C for 15 sec, 58° C for 30 sec and 72° C for 30 sec. The melting curve was evaluated for 1 cycle (95° C for 5 sec, 65° C for 30 sec and 95°C continuous). The last step of the program is the cooling for 1 cycle at 37° C for 5 min. Amplification efficiency (98% to 100%) for the primer was determined by amplification of cDNA dilution series of 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 ng per reaction. Primer specificity for PCR amplification was tested by melting curve analysis and agarose gel electrophoresis. Relative transcript

abundance was calculated and normalized with respect to 18S ribosomal RNA. Data shown represent mean values obtained from three biological replicates and two technical replicates and the error bars indicate the ± SE of the mean. For real time data analysis, we used the mathematic model proposed by Pfaffl (Pfaffl, 2001) to assess the relative expression levels of targets genes in comparison to a reference gene. The relative expression ratio (R) of target gene is calculated based on efficiency (E) and the CP deviation of a treated sample versus a control, and expressed in comparison to a reference gene:

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta CP_{\text{target}}\,(control\,-sample)}}{(E_{\text{ref}})^{\Delta CP_{\text{ref}}\,(control\,-sample)}}$$

E_{target} is the PCR efficiency of a target gene transcript; E_{ref} is the PCR efficiency of a reference gene transcript; ΔCP_{target} is the CP deviation of control – sample of the target gene transcript; ΔCP_{ref} is the CP deviation of control – sample of the reference gene transcript. Primers used for this study are listed in Table S-VII.

5.5 RESULTS

5.5.1 TRANSCRIPTOME ANALYSIS OF *mvp* MUTANT PLANTS USING MICROARRAY ANALYSIS

We identified *mvp* homozygous mutant plants by measuring the expression level of known markers for these plants (*Triticum monococcum VERNALIZATION 1* (*TmVRN1*), *Triticum monococcum FLOWERING LOCUS T like 1* (*TmFT1*) (Shimada *et al.*, 2009) and *Triticum monococcum PHYTOCHROME C* (*TmPHYC*) (Distelfeld et Dubcovsky, 2010). The expression level of all marker genes showed that these genes were not expressed in the *mvp* plants while their expression was normal in the wild type plants (Fig. 1a) allowing us to properly identify individual homozygous *mvp* plants. The identified mutant and WT plants plants were analysed using microarray analysis to identify the genes which expression is affected by the absence of *VRN1* and the other deleted genes. The analysis shows that, 368 probe sets IDs (genes) are differentially expressed in the *mvp* compared to the WT plants. From the 368 genes differentially regulated, we found that 263 genes distributed in six classes are up-regulated by 2 fold and more and 105 genes are down-regulated by 2 fold and less and distributed in five classes of genes (Figs. 1 b-d). The classes are: biotic stress related genes (class 1), transcription factors (class 2) sugar metabolism related genes (class 3), oxidative stress related genes (class 4) miscellaneous genes (class 5) and unknown genes (class 6) (Tables I, S-II - S-VI).

The biotic stress related genes class 1 are represented by 45 genes out the 368 genes (12,2%). These genes include genes encoding Flavanone hydroxylase (6,7 fold), Ice recrystallization inhibition (5,8 fold), Pathogenesis related 1a (5,4 fold), Thaumatin (5,1 fold) Chitinase (4,7 fold), Lipooxygenase (4 fold), Vacuolar defensine (3,1 fold), Dehydrin (2,4 fold), Endochitinase (2,3 fold) and Jasmonate-induced protein (down 7,2 fold) (Table I). From the 263 up-regulated genes, biotic

stress related genes represent (11,2%), the most important class of the specific genes classes (Classes 1-4) (Fig. 1c). The high up-regulation of the genes Flavanone hydroxylase and Lipoxygenase, involved in jasmonate synthesis, raises the hypothesis that jasmonates may accumulate in the *mvp* mutant plants (Table I).

An important part of the differentially regulated genes (class 6), 168 genes, out of the 368 (45,5%) are genes with unknown function (Table S-VI) indicating that many other aspects in this *mvp* mutant could be discovered in the future after identifying the function of these unknown genes. The micellaneous genes (class 5) contains 91 genes (24,7%). These genes are characterized but are involved in many aspects of plant development such as biological processes, molecular functions and cellular components. Genes that are highly up-regulated from this class include Apyrase, Chaperone protein, Agmatine coumaroyltransferase, BRASSINOSTEROID INSENSITIVE (BRI) 1-associated receptor kinase 1, Cold acclimation protein and Serine/threonine kinase-like protein (Table S-V). Multiple aspects of plant growth and development such as normal expression of a variety of developmental programs including cell elongation, vascular differentiation, seed germination, senescence and fertility are regulated by Brassinosteroids (BRs) and require an active BRASSINOSTEROID-INSENSITIVE 1 (BRI1) receptor serine/threonine kinase for hormone perception and signal transduction (Ehsan *et al.*, 2005). The fourth class represents oxidative stress related genes. These genes 21 genes out of 368 genes (5,7%) are all up-regulated genes, and encode Cytochrome c oxidase (up by 3,8 fold), Leucoanthocyanidin dioxygenase (3,3 fold), Peroxidase (3,0 fold) Cytochrome P450 (2,7 fold), Chloroplast lipocalin (2,4 fold) (Table S-IV). Genes from class 3 represent Sugar metabolism related genes (4,6%), 17 genes out of the 368 genes differentially regulated. This class contains genes encoding Glucomannan 4-beta-mannosyltransferase 1 (6,8 fold), Beta-glucanase (6,6 fold), Glucan endo-1,3-beta-glucosidase (3,9 fold), UDP-glucosyl transferase (3,4 fold) and Glucan endo-1,3-beta-glucosidase (3,4 fold) (Table S-III). The second class of genes contains transcription

factors and represents 7% of the differentially regulated genes (26 genes out of 368 genes). This class, contain genes encoding MADS-box, WRKY and CBFs transcription factors. Among genes that encode MADS-box transcription factors, many are highly down-regulated including the *VRN1* gene (down 108 fold) supporting the non-flowering status of these mutant *mvp* plants. Many genes that encode CBFs and WRKY transcription factors are up-regulated (Table S-II). Also we found the absence of flowering is not the only characteristics of the *mvp* plants, their roots are less developed compared to the wild type plants (Fig. S1). In plants, the involvement of soluble sugars in reactive oxygen species balance to response to oxidative stress is known (Couée *et al.*, 2006) while many WRKY transcription factors are involved in the regulation of plant defense responses (Li *et al.*, 2006).

Taken together these results suggest that mutant *mvp* induces not only the absence of flowering by the deletion of *VRN1* gene but also activates the molecular responses involved in the regulation of jasmonates biosynthesis and biotic stress responses(Table S-I).

5.5.2 VALIDATION OF THE MICROARRAY RESULTS

The microarray analysis shows that *VRN1* expression level was repressed by 108 folds in *mvp* plants compared to the WT plants. This analysis also reveals that two other genes were more repressed than *VRN1*. The most repressed gene, is repressed by 169 fold and corresponds to **Affymetrix Probe sets ID**: Ta.29481.1.S1_at, **GenBank Accession**: CK194207 and **UniProt Description**: Circumsporozoite protein or *TmCir*. The second most repressed (114 fold) corresponds to **Affymetrix Probe sets ID**: Ta.7832.1.S1_at, **GenBank Accession**: CA646083 and **GenBank Description**: Putative uncharacterized protein that we named *Triticum monococcum Uncharacterized Gene* (*TmUnG*). To confirm that these two genes are highly repressed, we measured their expression level using RT-PCR.

The results show that the *TmCir* and *TmUnG* are not expressed in the *mvp* mutant plants while a high expression level of these genes is found in WT plants (Fig. 2a). To determine if these two genes are not deleted in the *mvp* mutant plants, we extracted the genomic DNA from plant samples used for the microarray analysis to amplify the two genes by PCR. The results show that the coding sequence of the *TmCir* gene, the one repressed by 169-fold is not deleted by the bombardment although we cannot exclude a possible deletion of its promoter or an introduction of a mutation within the gene that affect its repression. On the other hand, the *TmUnG* repressed by 114-fold is deleted in the *mvp* mutant plants (Fig. 2b). BLAST search from GenBank database of Ta.29481.1.S1_at probe set ID from Affymetrix (*TmCir*) reveals a match with a clone named: WT010_A11 (Accession number AK334473) having a complete coding sequence cloned from wheat cv Chinese Spring (Kawaura *et al.*, 2009). Thus, more characterization can be done on this clone. Expression analysis using qRT-PCR confirmed the results from the microarray analysis of *TmVRN1*, *TmFT1* and *TmCir* by showing no detectable expression of these genes in *mvp* mutant plants while we found high expression in the WT plants (Fig. 2c and Table II). The qRT-PCR analysis also showed a high down-regulation of *TmCYS* expression in *mvp* plants, confirming its low expression by RT-PCR and its deletion as previously reported (Distelfeld et Dubcovsky, 2010). BLAST search of *TmCYS* gene from Affymetrix database of the wheat array reveals that, *TmCYS* is absent in the wheat array. This explains why we did not find TmCYS among the 368 genes that are differentially regulated in the microarray analysis. We also used *TmGI*, a gene that is implicated in the flowering process under photoperiod pathway and *TmOMT* a gene that encodes O-methyltransferase as controls. Their expression did not vary between *mvp* mutant and WT plants in the microarray analysis and this result is confirmed in the qRT-PCR analysis (Fig. 2c and Table II).

To further validate the microarray analysis, we selected several genes that are up-regulated in the *mvp* plants. The expression of the three genes (*TmLOX2*, *TmFLav*

and *TmPR1a*) that are associated with biotic stress (class 1 genes in Fig. 1c listed in Table I) were measured by qRT-PCR. *Lox2* encodes lipoxygenase 2, the enzyme that catalyses one step of the biosynthesis of jasmonic acid (JA) from membrane-derived linolenic acid (Beale et Ward, 1998 ; Farmer, Almeras et Krishnamurthy, 2003). Genes encoding *Lipoxygenase 2* and *Flavanone 3-hydroxylase* (*Flav*) are known to be highly induced by jasmonates (JA and MeJA) or in response to biotic stress (Edreva, 2005) while the gene encoding *Pathogenesis related* 1a (*PR1a*) is a salicylic acid responsive gene (Jaulneau *et al.*, 2010). Pathogenesis-related class 1 (PR1) proteins are defense factors ubiquitously synthesized by plants in response to pathogen infections. They contribute, directly or indirectly, to resistance to pathogen attack together with other defense proteins, such as β-1,3-glucanases, chitinases, and secondary metabolism enzymes including phytoalexin biosynthetic enzymes (Rivière *et al.*, 2008). The microarray analysis shows a high expression level of the three genes (*TmLOX2*, *TmFLav* and *TmPR1a*) in *mvp* plants compared to the WT plants (Tables I and S-I). The qRT-PCR analysis of these three genes confirms the expression profiles observed in the microarray analysis (Fig. 2d and Table S-I). Overall, RT-PCR and qRT-PCR analysis of ten genes (2 control non-regulated genes, 5 down-regulated genes and 3 up-regulated genes agree with the microarray results demonstrating the reliability of the global transcriptome analysis (Fig. 2). The microarray data, the Affymetrix Probe sets IDs and their descriptions are presented in Table II.

5.5.3 FREEZING TOLERANCE AND COR PROTEINS IN *mvp*, HETEROZYGOUS AND WILD TYPE PLANTS

To test if the *maintained vegetative phase* is associated with enhanced freezing tolerance, freezing test was performed in cold-acclimated and non-acclimated *mvp* mutant, heterozygous and wild type plants grown under long day

conditions. Non-acclimated plants from the three types of plants died after freezing at -6°C, while all the cold-acclimated plants survived at this temperature. Freezing tests of cold-acclimated plants at -8°C showed that 50 % and 58,33 % wild type plants died, 41,66 % and 37,55 %, heterogygous plants died and 25 % and 16,66 % *mvp* plants plants died for the first and second freezing tests respectively. Together, these results indicate that the *mvp* mutant plants have only a small improvement of freezing tolerance compared to the heterozygous and wild type plants respectively. To support the freezing tests results, we evaluated the expression of different COR proteins using western blot experiments. The western blot results show a slight increase accumulation of CORs proteins WCS 120 and WCS 19 in the *mvp* mutant plants compared to the WT plants that is in line with the marginal improvement of freezing tolerance in *mvp* mutant plants (Fig. 3). Taken together, these results demonstrate that the deletion of *VRN1* gene in einkorn wheat does not greatly affect their freezing tolerance or the accumulation of COR proteins compared to WT plants.

5.5.4 JASMONATES CONTENT IN *mvp* AND WILD TYPE EINKORN WHEAT PLANTS

The microarray analysis showed that the up-regulation of many genes associated with biotic stress and of specific genes involved in the synthesis of jasmonates (*Lipooxygenase* and *Flavanone hydroxylase*). These observations are similar to the molecular response associated with effects of jasmonate (Edreva, 2005) and suggest that *mvp* plants synthesized more jasmonate than the WT plants. To verify this hypothesis, we measured the content of jasmonic acid (JA) and methyl jasmonate (MeJA) in the *mvp* mutant, and WT einkorn wheat plants. The same plant material used for the microarray analysis was used to analyse the hormonal content. A comparable quantity of JA was found (148 ± 7ng g^{-1}FW) in WT plants and in *mvp* mutant plants (149 ± 4.57ng g^{-1}FW). On the other hand, MeJA was much higher in *mvp* mutant plants (72.7± 3.5ng g^{-1}FW) compared to the WT plants (11.5± 2.3ng g^{-}

^{1}FW). These results indicate a comparable JA content in the two types of plants while MeJA, the active molecule, accumulates approximately six fold in the *mvp* plants compared to the wild type einkorn wheat plants. This raises the question of the relationship between MeJA, up-regulation of PR genes, WRKY transcription factors, flowering genes and flowering delay in *mvp* plants.

5.5.5 EFFECT OF MeJA ON FLOWERING TIME

To test the hypothesis that MeJA is associated with the inability of the *mvp* plants to flower and its possible involvement in flowering delay, three week-old spring wheat plants that have the competence to flower, were treated with MeJA. The effect of MeJA on flowering time was quantified in two spring cultivars of hexaploid wheat (Manitou and Bounty) and one cultivar of spring *Triticum monococcum* of einkorn wheat using five different concentrations of MeJA (100, 150, 200, 300 and 450 μM). All plants treated with 150 μM including the einkorn wheat were very healthy and showed a significant delay in growth and flowering (Figs 4-S2). No significant difference was observed between the effects of 150 μM and 200 μM of MeJA. The highest MeJA concentration (300 and 450 μM) reduced significantly the growth and flowering time. However, the spikelets showed abnormalities, we thus chose 150 μM of methyl jasmonate for the exogenous treatment. The efficency of the treatment was assessed by measuring the endogenous jasmonate accumulation in the plants treated with 150 μM MeJA compared to non-treated plants cv Manitou wheat plants. The analysis indicates that the treated plants accumulate 805 ± 117ng g^{-1}FW of MeJA compared to 120.6 ± 11ng g^{-1}FW for non-treated plants. The JA content is 221 ± 4.9ng g^{-1}FW for treated plants and 28 ± 1.4ng g^{-1}FW for non-treated plants. This result indicates that the level of jasmonates in treated plants is approximately six times the level of jasmonates in non-treated plants confirming the efficiency of the treatment.

For phenotypical flowering time evaluation, three week-old plants cv Manitou and cv einkorn (Fig. 4-S2 a) were separated into two lots, one lot of plants was kept without treatment (Figs. 4-S2 b), and the other was sprayed with 150 µM MeJA every day for a period of 14 days (Figs. 4-S2 c). To show the phenotypical effect of methyl jasmonate in flowering (Figs 4-S2), pictures were taken before and after the methyl jasmonate treatment on both control and treated plants even two weeks after treatment (Figs 4-S2 d). These results show that methyl jasmonate induced a flowering delay in wheat in both cv Manitou and cv einkorn. To study the molecular and morphological effects of methyl jasmonate on wheat, we used only hexaploid wheat cv Manitou (Figs. 5-6). Plants were analysed at the end of the treatment. MeJA treatment inhibited shoot growth and reduced the development of root architecture, but had no effect on the emergence of news leaves. The percentage of flowering plants was calculated in both type of plants (Fig. 5a) and the treated plants produced 1.1 leaves more compared to the control non-treated plants as determined by FLN measurements (Fig. 5b) suggesting that *mvp* plants remained in the vegetative phase for a longer period. Flowering of treated plants was delayed by about two weeks (Figs. 4-5). In control plants, 100% of the plants flowered after 35 days while none of the MeJA treated plants flowered at this time. It took the MeJA treated plants 52 days before all the plants flowered (Fig. 5a). The treated plants showed an increase FLN compared to the control plants (Fig. 5b) confirming the flowering delay in the treated plants. These results indicate that MeJA act as growth and flowering inhibitor in wheat. To test if exogenous MeJA caused flowering delay by acting on flowering genes, we measured the expression level of several flowering genes in wheat (*TaVRN1*, *TaFT1* and *TaPHYC*) and the expression level of *TaCir*, the most repressed gene in the *mvp* mutant plants after the treatment with 150 µM MeJA. The qRT-PCR results show an increased expression level of all flowering genes in the first and second week in control non-treated plants. Interestingly, *TaCir* expression level shows a similar pattern of expression as the flowering genes suggesting its association with the flowering process in wheat. Furthermore, the

repression of *TaFT1* and *TaCir* is more pronounced indicating that these two genes are more sensitive to MeJA than *TaVRN1* and *TaPHYC* (Fig. 6) suggesting that MeJA may be acting through the repression of these two genes.

5.6 DISCUSSION

The mutant *mvp* was induced by nitrogen ion-beam treatment from the einkorn wheat (*Triticum monococcum*) and was identified by its inability to transit from the vegetative to the reproductive phase due to the deletion of *VRN1* gene (Shitsukawa *et al.*, 2007). However, our expression profiling showed that the production of *mvp* mutant plants caused not only the deletion of *VRN1* and its promoter but a larger deletion including an *UnG* gene (Fig. 2b). Our finding is in agreement with previous report (Distelfeld et Dubcovsky, 2010), where they reported that both *mvp* lines have larger deletions that include the genes *AGLG1*, *CYS*, *PHYC*, *VRN1* and possibly others. Taken together, this information suggests that the nitrogen ion-beam treatment used to produce the mutant *mvp* did not delet only the *VRN1* gene. A new study which put wheat's segregation population seed as transformation receptor from ion beam-mediated transformation strongly support such suggestion by showing an increased coefficient of variability of some characters such as plant height, grain's quality, spike length and also, the germination rate of different transformation combinations (Yunhong *et al.*, 2011). Thus this indicates that using ion beam-mediated transformation method to transform seeds could introduce many changes that are detected in a segregated population. In spite of this limitation, *mvp* mutants that lacks *VRN1* remains a valuable tool to understand the function and regulation of *VRN1* genes in flowering control. Yet there is no available mutant that lacks only *VRN1* and this mutant has only a few genes deleted. On a longer term, the information gathered with this mutant can eventually be validated by complementation experiments using the same mutant.

The microarray analysis of the *mvp* mutant plants show that the differentially regulated genes are associated with many processes in plant development such as response to stimulus multi-organism process, polysaccharide metabolism process and

cell wall macromolecule metabolic process, regulation of genes associated with transcription factor activity, hydrolase activity acting on glycosyl bound, iron ion binding and carbohydrate binding and the regulation of genes associated with cytoplasmic membrane-bound vesicules (Tables SI). The 368 genes differentially regulated from the microarray analysis were classified into 6 classes. The microarray analysis also showed two classes of genes that are associated with oxidative stress metabolism and sugar metabolism (Fig. 1c-d). Among up-regulated genes, we found genes that encodes CBFs transcriptions factors, cold acclimation induced protein and ice recrystallization inhibition protein. These results could help *mvp* plants to be more freezing tolerant than the WT plants. This is supported by the results from the freezing tolerance experiment and the accumulation of WCS 120 and WCS 19 proteins (Fig. 3). Our results support previous reports showing that the *mvp* plants showed higher freezing tolerance and increased transcript levels of several cold-induced *CBF* transcription factors and COR genes compare to the heterozygote (*Mvp/-*) plants (Dhillon *et al.*, 2010).

The majority of the highly up-regulated genes encode proteins that are related to biotic stress response such as flavanone hydroxylase, pathogenesis, chitinase, thaumatin, tipooxygenase, ice recrystallization inhibition protein, endochinase, dehydrin, tascular defensine (Table I). We found an up-regulation of several WRKY transcription factors (Table S-II). Some WRKY proteins are involved in the regulation of plant defense responses (Li *et al.*, 2006). Significant progress has been made on the characterization of WRKY proteins and data reported showed many are involved in the regulation of plant defense responses (Eulgem et Somssich, 2007 ; Li *et al.*, 2006). WRKY transcription factors have broad roles in orchestrating metabolic responses to biotic stress, and they represent potentially valuable tools for engineering metabolic changes that impact pathogen resistance (Naoumkina, He et Dixon, 2008). Members of the WRKY transcription factors family are involved in transcriptional regulation associated with plant immune responses (Eulgem et

Somssich, 2007) and development (Johnson, Kolevski et Smyth, 2002). Over-expression of *WRKY* genes in transgenic plants has shown that some are able to increase the production of PR proteins and to modulate resistance to phytopathogens (Li *et al.*, 2006). Complementation of the silencing by apoplastic treatment with a recombinant PR-1a protein restores largely the wild-type β-1,3-glucanase activity and callose phenotype. In addition, the immunolocalization of PR-1a in the sites of β-1,3-glucan deposition was found in wild-type tobacco plants. Together, these results are indicative of a function for PR-1a in regulation of enzymatic activity of extracellular b-(1/3)-glucanases (Rivière *et al.*, 2008). Also, it is known that jasmonates activate plant defense mechanisms in response to insect-driven wounding, various pathogens, and environmental stresses, such as drought, low temperature, and salinity (Wasternack et Hause, 2002). A study shows that genes that are up-regulated by MeJA treatment include those involved in jasmonate biosynthesis, secondary metabolism, cell-wall formation, and those encoding stress protective and defense proteins (Cheong et Choi, 2003).

Stress situations are related to important changes in reactive oxygen species balance. Soluble sugars, especially sucrose, glucose, and fructose, play a central role as nutrient and metabolite signalling molecules that activate specific or hormone crosstalk transduction pathways, thus resulting in important modifications of gene expression patterns in response to a number of stresses (Couée *et al.*, 2006). All these links place soluble carbohydrates in a pivotal role in the pro-oxidant and antioxidant balance. Thus this suggests involvement of soluble sugars in mechanisms that prevent de-regulation of reactive oxygen species production. Jasmonates and salicylates hormones act both locally and systemically to orchestrate the plant's defense signaling network through the activation of transcription factors (Kidd *et al.*, 2009). Jasmonates were shown to activate plant defense mechanisms by inducing expression of WRKY transcription factors in response to biotic stress such as insect-driven wounding, various pathogens, and environmental stresses, such as drought, low

temperature, and salinity (Cheong et Choi, 2003 ; Kim *et al.*, 2010 ; Li *et al.*, 2006). Furthermore, jasmonate was shown to either promote in which delay flowering depends up on its concentration (Krajnčič, Krist et Janžekovič, 2006).

The microarray and molecular results obtained in this study and the results reported before (Distelfeld and Dubcovsky, 2010) confirm that not only *VRN1* was deleted when these *mvp* plants were produced. More importantly, the phenotype observed in *mvp* plants could be associated not only on flowering process but also other aspects of plant development such as roots development and cellular elongation. In the *mvp* mutant plants, the microarray analysis shows the up-regulation of genes related to all these processes (Table S-I). The high up-regulation of the *Flavanone hydroxylase* and *Lipooxygenase* in addition to these findings suggest a regulation of jasmonate biosynthesis in the *mvp* mutant plants and led us to quantify jasmonates (JA and MeJA) in *mvp* mutant and WT plants. JA and MeJA have been found to occur naturally in a wide range of higher plants. Methyl jasmonate (MeJA) is derived from jasmonic acid and the reaction is catalyzed by S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase and has been identified as a vital cellular regulator that mediates diverse cellular responses such as defense and developmental pathways such as seed germination, root growth, flowering, fruit ripening and senescence (Cheong et Choi, 2003). The two forms of jasmonate are presumably inter-convertible within plants by JA methyltransferase(s) (Seo *et al.*, 2001) and MeJA esterase(s) (Stuhlfelder, Mueller et Warzecha, 2004). Understanding how MeJA biogenesis is regulated at the molecular level, and how it relates to jasmonate-responsive gene activation, is still incomplete. Plants scent methyl jasmonate (MeJA) as a vital cellular regulator that mediates diverse developmental processes and defense responses against biotic and abiotic stresses and the pleiotropic effects of MeJA have raised numerous questions about its regulation for biogenesis and mode of action (Cheong et Choi, 2003).

Our results showed that the *mvp* plants synthesized high levels of methyl jasmonate (MeJA) compared to wild type plants. These findings in addition to the non-flowering pheneotype of the *mvp* mutant plants, suggested that MeJA could be involved in regulating the flowering process in wheat. Results in figure 4 shows that MeJA delays flowering in a signifacant manner. This delay in flowering is associated with important changes in the expression of the flowering genes with the most important effect on *FT1* and an important decrease in *VRN1* and *PHYC*. *TaCir* expression profile showed similar pattern to the flowering gene suggesting that it may be involved in the regulation of flowering in wheat (Fig. 6). The high amount of MeJA in *mvp* mutant plants may thus contribute to the down-regulation of *Cir* and *FT1* expression even the *PHYC* deletion could also have a role in the down-regulation of *FT1*. This raises the hypothesis that MeJA could interact with *FT1* and *Cir* genes. In the case of *FT1* gene, such hypothesis is supported by sequence analysis of *FT1* promoter showing the presence of MeJA binding element. Thus the low level of *FT1* gene expression in *mvp* plants could be caused by its repression by MeJA which is biosynthesized in high amount in these plants.

Conclusion

In this study, our results showed that the *mvp* mutant plants induced the up-regulation of several genes related to plant defense mechanisms in response to the deletions caused by the ion beam-mediated transformation. More importantly, our data revealed that *mvp* plants accumulated a high level of methyl jasmonate (MeJA) compared to the wild type einkorn wheat plants. In wheat, our data also demonstrate that MeJA causes flowering delay by repressing *FT1* gene expression.

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Figure 1: Molecular characterization and microarray analysis of the maintained vegetative phase (*mvp*). Total RNA was extracted from whole aerial parts and analysed by RT-PCR. Each replicate (R1, R2 and R3) was obtained from five mutants plants (*mvp)* or from three control plants (WT). **a)** Relative expression level of gene markers of maintained vegetative phase (*mvp*) plants and control (WT) plants were analysed by RT-PCR to validate the *mvp* mutant. The 18S *TaRNA* was used for load control. The experiments were repeated 3 times with the same result. **b)** Volcano plots illustrating the log2 fold changes in gene expression differences between *mvp* and WT plants. Probesets with statistically different expression (P value ≤ 0.05) and fold changes of ≥ 2 fold (263) are shown in the upper right panel and Probe sets with ≤ -2 fold (105) are shown in the upper left panel. Pie charts of differentially regulated genes in *mvp* mutant plants compared to wild type plants derived from the microarray analysis: **c)** Up-regulated genes; **d)** down-regulated genes. The numbers **(1- 6)** represent groups of differentially regulated genes.

Figure 2: Relative expression level of selected genes for validation of the microarray analysis of *mvp* plants and wild type control plants analysed by RT-PCR and qRT-PCR. Total RNA was extracted from whole plants and analysed by qRT-PCR. Each replicate (R1, R2 and R3) was obtained from five mutants plants (mvp) and from three control plants (WT). **a)** Validation of *TmUnG* and *TmCir* expression by RT-PCR. The 18S *TaRNA* was used for load control. **b)** Genomic DNA was extracted from the same plant samples used for the microarray analysis and were tested for PCR amplification to analyse the possible deletion of the two most repressed genes from the array analysis (*TmUnG* repressed 114 times and *TmCir* repressed 169 times). The 18S *TaRNA* is used for load control. The experiments were repeated with 3 different biological replicates with the same result. Validation of the selected genes using qRT-PCR: **c)** down-regulated and control genes; **d)** up-regulated and control genes. Relative transcript abundance was calculated and normalized with respect to 18S *TaRNA* for the qRT-PCR experiment. Data shown represent mean

values obtained from three biological replicates and the error bars indicate the ± SE of the mean.

Figure 3: Expression level of CORs proteins analysed by western blot. After 3 weeks of germination at 20°C under long day photoperiod (LD), plants were cold-acclimated for 14 days under LD photoperiod at 4°C. Equal amounts of total soluble proteins from whole plant tissues were analysed. Proteins were separated on a 12% SDS–PAGE and transferred to a polyvinylidene difluoride membrane, and an anti-WCS120, anti-Wcor410, anti-WCS19 and anti-TaIRI antibodies were used to detect the corresponding proteins. Rubisco was used as load control. The experiment was repeated four times with the same results.

Figure 4: Effect of methyl jasmonate treatment on plant development in wheat cv Manitou. a) After 3 weeks of germination at 20°C under long-day photoperiod (LD), **b**) non-treated spring wheat plants (cv Manitou) were kept under LD conditions at 20°C and **c**) treated plants were sprayed with 150 µM of methyl jasmonate everyday for 2 weeks under the same growth conditions. Pictures were taken for 21 day-old control plants immediately after MeJA treatment: 2 weeks of control and treated plants and **d)** two weeks after the end of MeJA treatment for control and treated plants.

Figure 5: Effect of methyl jasmonate treatment on flowering and Final Leaf Number in hexaploid wheat plants cv Manitou (*Triticum aestivum*). a) Plants were sprayed with 150 µM of methyl jasmonate everyday between 21 to 35 days for threated plants and the percentage of flowering plants was measured for both treated and non-treated plants. **b)** Final Leaf Number. Results were expressed as value ± SEM of three different experiments. Comparison between groups and analysis for differences between means of control and treated groups were performed using

ANOVA followed by the post-hoc test Newman–Keuls. The threshold for statistical significance was: *: $p < 0.05$.

Figure 6: Effect of methyl jasmonate treatment on the expression of flowering associated genes (*TaVRN-A1*, *TaFT1-A1*, *TaPHYC-A* and *TaCir*). Relative expression was analysed qRT-PCR after treating the spring wheat cultivar (cv Manitou) with 150 µM of methyl jasmonate for two weeks everyday. The 18S *TaRNA* is used as load control. After 3 weeks of germination at 20°C under LD conditions, control non-treated spring wheat (cv Manitou) plants (Ctrl) were kept under LD conditions at 20°C for 21 days and treated plants were sprayed with 150 µM of methyl jasmonate everyday for 2 weeks. Total RNA was extracted from areal parts and analysed by qRT-PCR. Data shown represent mean values obtained from three biological replicates and two technical replicates and the error bars indicate the ± SE of the mean.

Table 5.1 *mvp* wheat plant class 1 regulated genes (biotic stress related genes) identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
Ta.3976.2.S1_x_at	CA679100	Triticum aestivum flavanone 3-hydroxylase mRNA, partial cds	6,713
Ta.18574.1.A1_x_at	CK196896	Ice recrystallization inhibition protein 4 (Fragment) n=1 Tax=Triticum aestivum RepID=B9VR51_WHEAT	5,807
Ta.3976.1.S1_at	CA678526	Triticum aestivum flavanone 3-hydroxylase mRNA, partial cds	5,510
Ta.27327.1.S1_x_at	BT009360	Pathogenesis-related 1a n=1 Tax=Triticum monococcum RepID=Q3S4I4_TRIMO	5,467
Ta.959.1.S1_at	CA721939	Thaumatin-like protein n=3 Tax=Triticum RepID=Q41584_WHEAT	5,147
Ta.221.1.S1_at	AF112963	Chitinase II n=1 Tax=Triticum aestivum RepID=Q9XEN3_WHEAT	4,725
Ta.27762.1.S1_x_at	AF384146	Pathogenesis-related protein 1A/1B n=10 Tax=Triticeae RepID=PR1A_HORVU	4,714
Ta.24501.1.S1_at	CD863039	Pathogenesis-related protein 1A/1B n=10 Tax=Triticeae RepID=PR1A_HORVU	4,689
Ta.22619.1.S1_at	CA687670	Pathogenesis-related protein 10 n=1 Tax=Hordeum vulgare RepID=Q84QC7_HORVU	4,186
Ta.22619.1.S1_x_at	CA687670	Pathogenesis-related protein 10 n=1 Tax=Hordeum vulgare RepID=Q84QC7_HORVU	4,053
TaAffx.104812.1.S1_s_at	BJ223744	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	4,031
TaAffx.28302.2.S1_at	CA695754	Dirigent-like protein n=2 Tax=Oryza sativa RepID=Q53NP6_ORYSJ	4,006
Ta.1967.1.S1_x_at	CK152466	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	3,927
Ta.1967.2.A1_x_at	AJ614579	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	3,691
Ta.23322.2.S1_at	CA640491	Thaumatin-like protein TLP8 n=1 Tax=Hordeum vulgare RepID=Q946Y8_HORVU	3,672
Ta.224.1.S1_at	AF112966	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	3,375
TaAffx.107480.1.S1_at	CA679967	Ice recrystallization inhibition protein 5 n=1 Tax=Deschampsia antarctica RepID=C0L702_DESAN	3,357
TaAffx.128595.1.S1_at	CK216241	Putative vacuolar defense protein n=2 Tax=Triticum aestivum RepID=Q6PWL8_WHEAT	3,294
Ta.2709.1.S1_s_at	CK166154	Defensin-like protein 2 n=1 Tax=Triticum aestivum RepID=DEF2_WHEAT	3,188
Ta.7022.1.S1_s_at	BJ281221	Phenylalanine ammonia-lyase n=5 Tax=Poaceae RepID=Q7F929_ORYSJ	2,868
TaAffx.137429.1.S1_at	CA610138	Dehydrin 5 (Fragment) n=1 Tax=Hordeum vulgare subsp. spontaneum RepID=Q6V7D2_HORSP	2,645
Ta.7022.2.S1_at	BF199967	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,604
Ta.25077.1.A1_at	BQ161103	Ice recrystallization inhibition protein 2 n=1 Tax=Triticum aestivum RepID=Q56B89_WHEAT	2,580
Ta.7022.2.S1_x_at	BF199967	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,569
Ta.21556.1.S1_at	CA684533	Protein WIR1B n=1 Tax=Triticum aestivum RepID=WIR1B_WHEAT	2,505
Ta.25026.1.S1_at	BQ804965	Dehydrin n=1 Tax=Triticum turgidum subsp. durum RepID=Q5CAQ2_TRITU	2,504
Ta.21768.1.S1_x_at	CA701727	Ice recrystallization inhibition protein 7 n=1 Tax=Deschampsia antarctica RepID=C0L704_DESAN	2,475
Ta.16472.1.S1_s_at	CA606887	Pathogenesis-related protein n=1 Tax=Hordeum vulgare RepID=P93181_HORVU	2,418
Ta.12663.1.S1_at	CK197682	Ice recrystallization inhibition protein 1 n=1 Tax=Triticum aestivum RepID=Q56B90_WHEAT	2,334
TaAffx.128418.43.S1_at	BJ252866	Endochitinase n=5 Tax=Pooideae RepID=Q41539_WHEAT	2,324
Ta.28659.3.S1_x_at	CA689419	Putative protease inhibitor n=1 Tax=Hordeum vulgare RepID=Q96465_HORVU	2,317
Ta.22678.1.A1_a_at	CK214868	Chitinase 1 n=2 Tax=Andropogoneae RepID=B4FBN8_MAIZE	2,294

TaAffx.45277.1.S1_x_at	BJ231180	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,263
Ta.2278.2.S1_a_at	CK196331	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	2,254
Ta.2278.3.S1_x_at	CD490414	Chitinase II n=1 Tax=Triticum aestivum RepID=Q9XEN3_WHEAT	2,234
Ta.27389.2.S1_x_at	BJ297034	Defensin-like protein 2 n=1 Tax=Triticum aestivum RepID=DEF2_WHEAT	2,202
Ta.2278.2.S1_x_at	CK196331	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	2,086
Ta.12820.1.S1_at	CK215415	Defensin n=1 Tax=Triticum turgidum subsp. durum RepID=C9E1C6_TRITU	-2,245
Ta.28133.1.A1_s_at	CA636835	Dirigent-like protein, expressed n=2 Tax=Oryza sativa RepID=Q2R0I1_ORYSJ	-2,438
Ta.7963.2.S1_x_at	CK215257	Dirigent-like protein, expressed n=2 Tax=Oryza sativa RepID=Q2R0I1_ORYSJ	-2,584
Ta.7388.2.S1_x_at	BU672305	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-2,636
Ta.7108.1.S1_at	CF134173	Ice recrystallization inhibition protein 2 n=1 Tax=Triticum aestivum	-3,427
TaAffx.98064.1.A1_at	BQ168859	Ice recrystallization inhibition protein 3 n=1 Tax=Deschampsia antarctica	-4,022
Ta.7388.1.S1_at	BJ320233	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-7,187
Ta.7388.2.S1_a_at	BU672305	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-7,230

Legend: According to Affymetrix Gene Chip® wheat genome array of the 45 biotic stress probe sets IDs differentially regulated (see Fig. 1) complemented with BLAST results showing the Genbank accession number, UniProt or NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

Table 5.2 *mvp* wheat plant of validated genes and their fold change from the microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
Ta.3976.2.S1_x_at	CA679100	Triticum aestivum flavanone 3-hydroxylase mRNA, partial cds	6,713
Ta.27327.1.S1_x_at	BT009360	Pathogenesis-related 1a n=1 Tax=Triticum monococcum RepID=Q3S4I4_TRIMO	5,467
TaAffx.104812.1.S1_s_at	BJ223744	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	4,031
Ta.10215.1.S1_at	AY679115.1	Triticum aestivum gigantean 3 (TaGI3) mRNA, complete cds	-0,035
Ta.11017.1_A1_at	B4ERX7	Triticum aestivum O-methyltransferase	-0,447
Ta.30640.1.S1_at	CD861747	Triticum aestivum flowering locus T mRNA, complete cds or VRN3	-3,250
Ta.28005.1.A1_at	CD862101	Phytochrome C (Fragment) n=1 Tax=Hordeum vulgare RepID=Q945T7_HORVU	-16,267
TaAffx.143995.17.S1_s_at	AY188331	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-108,744
TaAffx.85922.1.S1_s_at	CA618396	Putative uncharacterized protein Sb01g007930 n=2 Tax=Poaceae RepID=C5X0B2_SORBI (UnG)	-114,866
Ta.29481.1.S1_at	CK194207	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-169,064

Legend: According to Affymetrix Gene Chip® wheat genome array of the 10 Probe sets IDs used in RT-PCR and qRT-PCR experiments to validate microarray expression profiling analysis complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

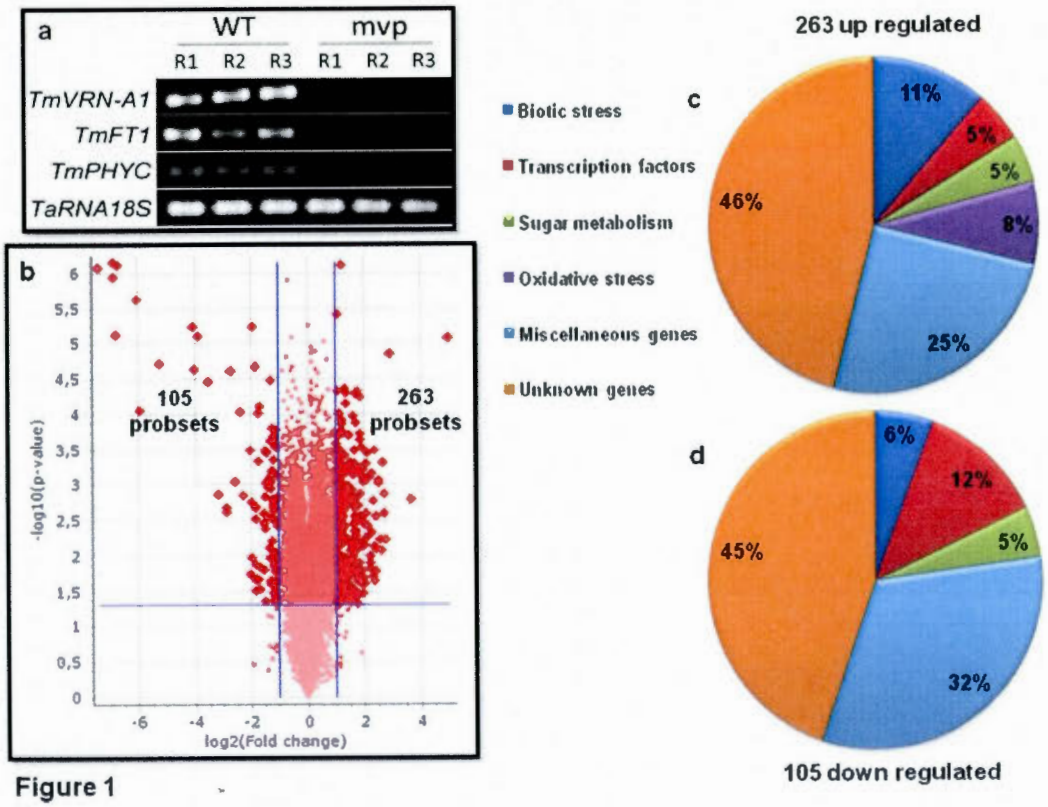

Figure 1

Figure 5.1 Molecular characterization and microarray analysis of the maintained vegetative phase (*mvp*)

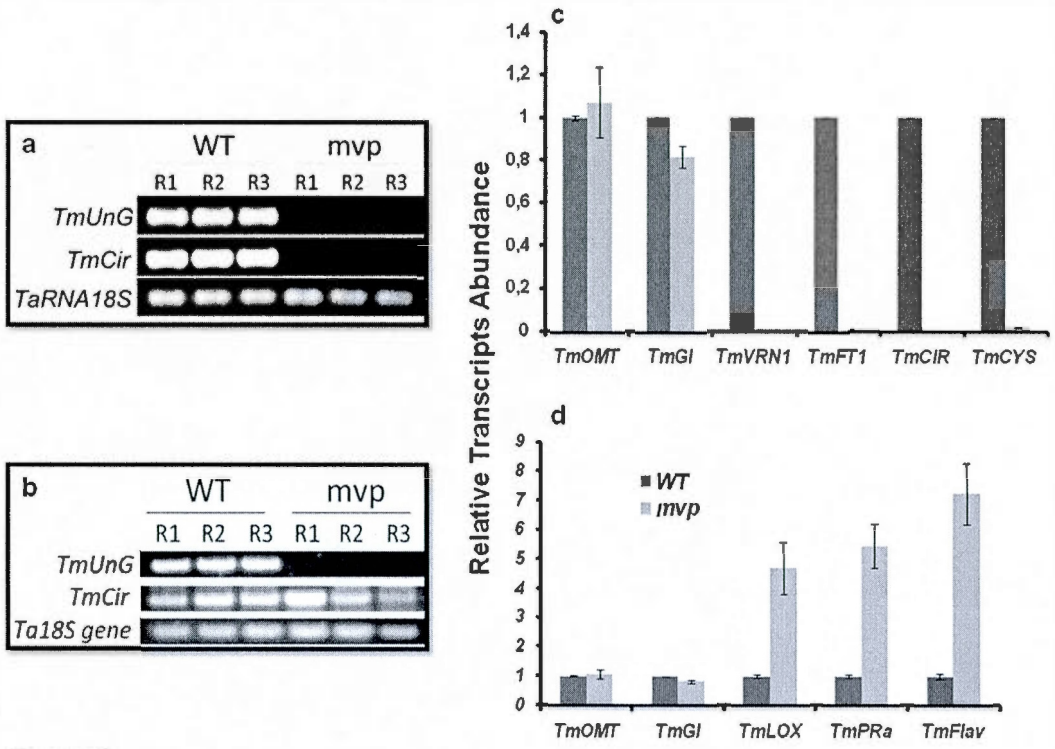

Figure 2

Figure 5.2 Relative expression level of selected genes for validation of the microarray analysis of *mvp* plants and wild type control plants analysed by RT-PCR and qRT-PCR

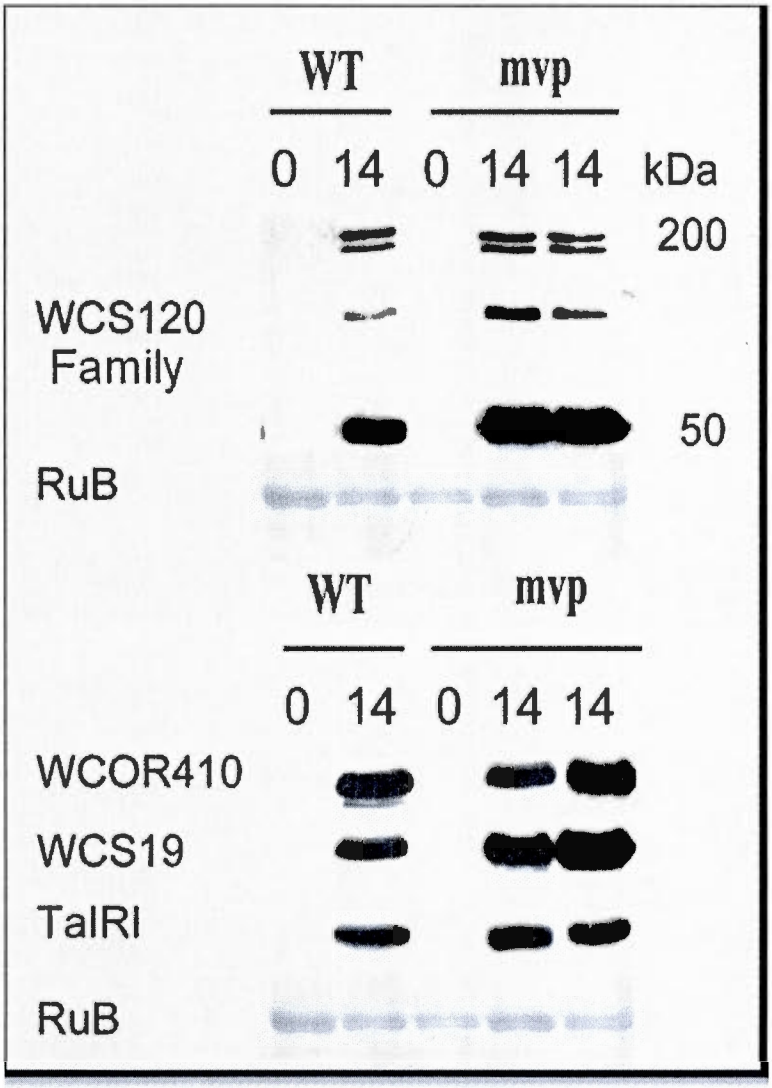

Figure 3

Figure 5.3 Expression level of CORs proteins analysed by western blot

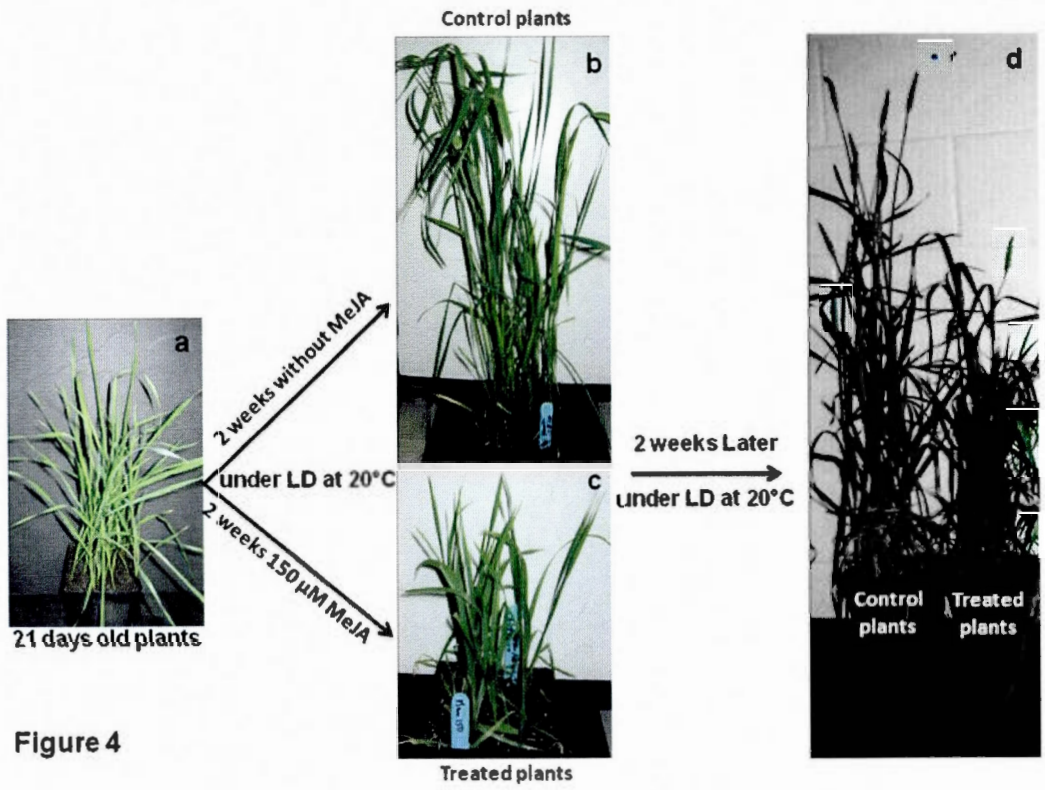

Figure 5.4 Effect of methyl jasmonate treatment on plant development in wheat cv Manitou

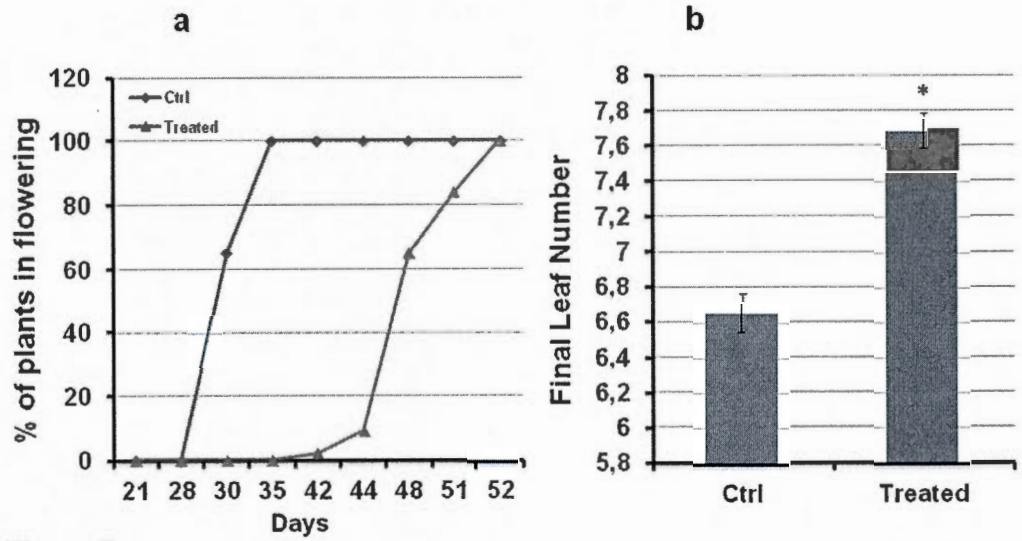

Figure 5

Figure 5.5 Effect of methyl jasmonate treatment on flowering and Final Leaf Number in hexaploid wheat plants cv Manitou (*Triticum aestivum*)

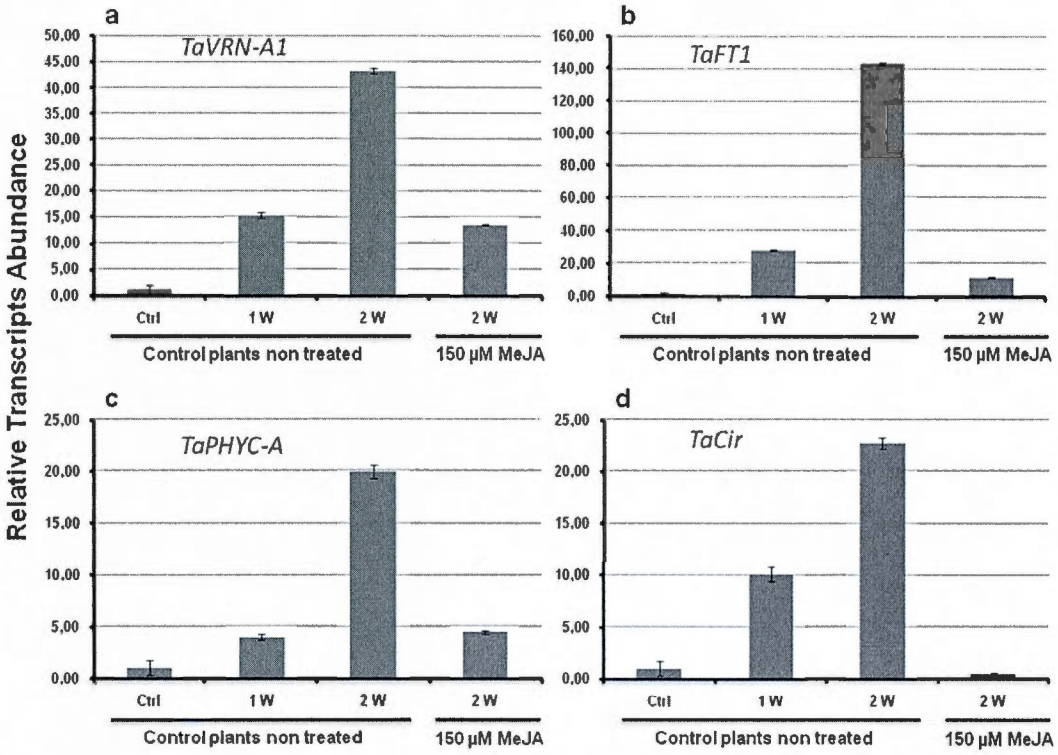

Figure 6

Figure 5.6 Effect of methyl jasmonate treatment on the expression of flowering associated genes (*TaVRN-A1*, *TaFT1-A1*, *TaPHYC-A* and *TaCir*)

Table S5. 1 *mvp* wheat plant regulated genes identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change	Genes Classes
TaAffx.57571.1.S1_at	CA626944	Apyrase n=1 Tax=Lolium perenne RepID=B9U140_LOLPR	31,363	5
Ta.8447.1.S1_a_at	CA669038	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8AKC8_ORYSI	12,977	6
TaAffx.70677.1.S1_at	CA701525	Chaperone protein dnaJ n=2 Tax=Andropogoneae RepID=B4FBY1_MAIZE	7,753	5
TaAffx.107485.1.S1_at	CA699183	Glucomannan 4-beta-mannosyltransferase 1 n=3 Tax=Oryza sativa RepID=CSLA1_ORYSJ	6,833	3
Ta.3976.2.S1_x_at	CA679100	Triticum aestivum flavanone 3-hydroxylase mRNA, partial cds	6,713	1
Ta.26048.1.S1_x_at	CD454944	Beta-glucanase n=1 Tax=Hordeum vulgare RepID=Q7M1K2_HORVU	6,647	3
TaAffx.89610.1.S1_at	AL823263	Agmatine coumaroyltransferase n=1 Tax=Zea mays RepID=B6ST92_MAIZE	6,540	5
Ta.27314.1.S1_at	BT009398	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays	6,180	5
Ta.19723.1.S1_at	CA657361	wlm0.pk0034.h7 wlm0 Triticum aestivum cDNA clone wlm0.pk0034.h7 5' end	6,056	6
Ta.18574.1.A1_x_at	CK196896	Ice recrystallization inhibition protein 4 (Fragment) n=1 Tax=Triticum aestivum RepID=B9VR51_WHEAT	5,807	2
TaAffx.109794.1.S1_s_at	CA668708	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8AKC8_ORYSI	5,763	6
Ta.8902.2.S1_at	CA640147	Taxane 13-alpha-hydroxylase n=1 Tax=Zea mays RepID=B6SYR0_MAIZE	5,532	5
Ta.3976.1.S1_at	CA678526	Triticum aestivum flavanone 3-hydroxylase mRNA, partial cds	5,510	1
Ta.27327.1.S1_x_at	BT009360	Pathogenesis-related 1a n=1 Tax=Triticum monococcum RepID=Q3S4I4_TRIMO	5,467	1
Ta.62.1.S1_x_at	BM136002	Os01g0382000 protein n=5 Tax=Oryza sativa RepID=Q7F2P0_ORYSJ	5,324	6
TaAffx.110724.1.S1_at	CA655297	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B8A3M2_MAIZE	5,219	6
Ta.28233.1.S1_at	CA599187	Putative uncharacterized protein Sb10g024350 n=1 Tax=Sorghum bicolor RepID=C5Z653_SORBI	5,214	6
Ta.23271.1.S1_s_at	CA680274	wlm24.pk0004.a5 wlm24 Triticum aestivum cDNA clone wlm24.pk0004.a5 5' end	5,156	6
Ta.959.1.S1_at	CA721939	Thaumatin-like protein n=3 Tax=Triticum RepID=Q41584_WHEAT	5,147	1
Ta.8356.1.S1_at	BQ161783	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKT6_ORYSI	5,002	6
Ta.30765.1.S1_at	CN011347	Putative uncharacterized protein Sb01g037040 n=1 Tax=Sorghum bicolor RepID=C5X1U8_SORBI	4,935	6
Ta.221.1.S1_at	AF112963	Chitinase II n=1 Tax=Triticum aestivum RepID=Q9XEN3_WHEAT	4,725	1
Ta.27762.1.S1_x_at	AF384146	Pathogenesis-related protein 1A/1B n=10 Tax=Triticeae RepID=PR1A_HORVU	4,714	1
Ta.24501.1.S1_at	CD863039	Pathogenesis-related protein 1A/1B n=10 Tax=Triticeae RepID=PR1A_HORVU	4,689	1
Ta.26917.1.S1_at	CD452828	MADS-box transcription factor TaAGL41 n=2 Tax=Triticum aestivum RepID=Q1G168_WHEAT	4,600	2
TaAffx.53867.1.S1_at	CA688277	wlm96.pk039.j13 wlm96 Triticum aestivum cDNA clone wlm96.pk039.j13 5' end	4,499	6
Ta.23271.2.S1_a_at	CA677139	wlm12.pk0009.c12 wlm12 Triticum aestivum cDNA clone wlm12.pk0009.c12 5' end	4,484	6
TaAffx.61466.1.S1_at	BJ251396	Putative uncharacterized protein Sb03g037575 n=1 Tax=Sorghum bicolor RepID=C5XN35_SORBI	4,472	6
Ta.28917.2.S1_a_at	CA637102	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	4,341	5
Ta.25531.1.A1_at	CD373817	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	4,302	5
TaAffx.97767.1.A1_at	CA721990	Putative uncharacterized protein Sb01g033530 n=1 Tax=Sorghum bicolor RepID=C5WXP8_SORBI	4,222	6
Ta.21340.1.S1_a_at	CA683606	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=Q6ZKP5_ORYSJ	4,203	6
Ta.8356.1.S1_s_at	BQ161783	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKT6_ORYSI	4,191	6

Ta.22619.1.S1_at	CA687670	Pathogenesis-related protein 10 n=1 Tax=Hordeum vulgare RepID=Q84QC7_HORVU	4,186	1
TaAffx.7302.1.S1_at	CA662601	Os01g0678000 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q5QM71_ORYSJ	4,055	6
Ta.22619.1.S1_x_at	CA687670	Pathogenesis-related protein 10 n=1 Tax=Hordeum vulgare RepID=Q84QC7_HORVU	4,053	1
TaAffx.104812.1.S1_s_at	BJ223744	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	4,031	1
TaAffx.28302.2.S1_at	CA695754	Dirigent-like protein n=2 Tax=Oryza sativa RepID=Q53NP6_ORYSJ	4,006	1
TaAffx.27177.1.S1_at	CA680302	Putative uncharacterized protein Sb09g004960 n=1 Tax=Sorghum bicolor RepID=C5Z115_SORBI	3,985	6
TaAffx.15327.1.S1_at	AJ610775	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,959	3
Ta.23165.2.S1_x_at	CA667728	Triticum aestivum clone wlsu2.pk0001.h3:fis, full insert mRNA sequence	3,958	6
Ta.1967.1.S1_x_at	CK152466	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	3,927	1
TaAffx.111759.3.S1_s_at	CA660613	wlm1.pk0023.g12 wlm1 Triticum aestivum cDNA clone wlm1.pk0023.g12 5' end	3,918	6
Ta.23165.3.S1_x_at	CA669496	Triticum aestivum clone wlsu2.pk0001.h3:fis, full insert mRNA sequence	3,912	6
TaAffx.1074.1.S1_at	CK212638	Cytochrome c oxidase subunit 1 n=9 Tax=Aphidomorpha RepID=Q69HZ8_9HEMI	3,884	4
TaAffx.43393.1.S1_at	BQ482808	Os02g0102900 protein n=4 Tax=Poaceae RepID=Q6ZFJ9_ORYSJ	3,861	6
Ta.192.1.S1_at	U32431	Putative uncharacterized protein n=1 Tax=Triticum aestivum RepID=Q41523_WHEAT	3,853	6
Ta.14779.1.S1_at	CA681945	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	3,794	6
Ta.27503.1.A1_at	CA659276	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8B370_ORYSI	3,794	6
Ta.18720.1.S1_x_at	BJ288588	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	3,753	5
Ta.12198.1.A1_at	BQ171803	WHE1659-1662_P05_P05ZT Wheat heat stressed flag leaf cDNA library Triticum aestivum	3,723	6
Ta.8902.1.S1_at	BQ162604	Taxane 13-alpha-hydroxylase n=1 Tax=Zea mays RepID=B6SYR0_MAIZE	3,703	5
Ta.1967.2.A1_x_at	AJ614579	Lipoxygenase 2.1, chloroplastic n=1 Tax=Hordeum vulgare RepID=LOX21_HORVU	3,691	1
Ta.9430.1.S1_at	AJ614438	Putative uncharacterized protein Sb10g004170 n=1 Tax=Sorghum bicolor RepID=C5Z4H2_SORBI	3,685	6
Ta.23322.2.S1_at	CA640491	Thaumatin-like protein TLP8 n=1 Tax=Hordeum vulgare RepID=Q946Y8_HORVU	3,672	1
Ta.19805.2.S1_a_at	CA683372	Os04g0168400 protein n=3 Tax=Oryza sativa RepID=B7F8V8_ORYSJ	3,659	6
Ta.12118.1.S1_a_at	CA698971	Putative uncharacterized protein n=1 Tax=Zea mays RepID=C0PFC3_MAIZE	3,606	6
TaAffx.97737.1.A1_at	CA722456	Triticum aestivum cDNA, clone: WT006_G18, cultivar: Chinese Spring	3,559	6
TaAffx.122333.1.S1_at	CA725295	Putative uncharacterized protein Sb03g045090 n=1 Tax=Sorghum bicolor RepID=C5XH43_SORBI	3,539	6
TaAffx.26668.1.S1_at	CA686407	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	3,528	6
TaAffx.122374.1.A1_at	BE517594	CBFIVa-2.2 (Fragment) n=1 Tax=Triticum aestivum RepID=A0MPK8_WHEAT	3,525	2
Ta.22565.1.S1_at	BT009372	UDP-glucosyl transferase n=1 Tax=Triticum aestivum RepID=C5HUX8_WHEAT	3,453	3
TaAffx.13303.1.S1_at	BG909514	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKZ0_ORYSI	3,437	6
TaAffx.132143.1.S1_s_at	CK212211	Cyanate hydratase n=3 Tax=Oryza sativa RepID=CYNS_ORYSJ	3,432	5
Ta.520.1.S1_at	BE604553	WHE1413-1416_P07_P07ZS Wheat drought stressed leaf cDNA library Triticum aestivum	3,429	6
Ta.25531.2.A1_x_at	CA680295	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	3,426	5
TaAffx.108556.1.S1_at	CA692789	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B4FS23_MAIZE	3,396	6
TaAffx.24475.1.S1_at	CA718969	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,382	3
TaAffx.100029.1.S1_at	CA678048	Leucoanthocyanidin dioxygenase n=1 Tax=Zea mays RepID=B6U9L0_MAIZE	3,377	4

Ta.5766.1.S1_at	BJ289079	Os03g0218400 protein n=5 Tax=Poaceae RepID=Q10PW9_ORYSJ	3,376	6
Ta.224.1.S1_at	AF112966	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	3,375	1
Ta.7711.1.A1_at	BQ161212	Putative uncharacterized protein Sb08g017560 n=1 Tax=Sorghum bicolor RepID=C5YPJ9_SORBI	3,359	6
TaAffx.107480.1.S1_at	CA679967	Ice recrystallization inhibition protein 5 n=1 Tax=Deschampsia antarctica RepID=C0L702_DESAN	3,357	2
Ta.231.1.S1_x_at	AF079526	PR17c n=2 Tax=Triticeae RepID=A7YA60_HORVD	3,356	6
Ta.9220.1.S1_a_at	CK210556	Putative phenylalanine ammonia-lyase (Fragment) n=1 Tax=Hordeum vulgare	3,352	5
TaAffx.70601.1.S1_at	BQ802878	Triticum aestivum cultivar 92R137 PDR-type ABC transporter (PDR1)	3,344	5
TaAffx.26815.1.S1_at	CA684496	BLN1-2 n=3 Tax=Hordeum vulgare subsp. vulgare RepID=B8X453_HORVD	3,336	6
TaAffx.128798.2.S1_x_at	CA695322	Putative uncharacterized protein Sb08g017540 n=1 Tax=Sorghum bicolor RepID=C5YPJ7_SORBI	3,319	6
Ta.8232.1.A1_at	BQ161629	PDR-type ABC transporter n=2 Tax=Triticum aestivum RepID=B9UYP3_WHEAT	3,309	5
TaAffx.128595.1.S1_at	CK216241	Putative vacuolar defense protein n=2 Tax=Triticum aestivum RepID=Q6PWL8_WHEAT	3,294	1
TaAffx.97211.1.S1_at	AJ614654	O-methyltransferase n=1 Tax=Triticum aestivum RepID=B4ERX7_WHEAT	3,268	5
Ta.3869.1.S1_at	CA674403	Os08g0127100 protein n=6 Tax=Poaceae RepID=Q6ZK52_ORYSJ	3,266	6
TaAffx.55188.1.S1_at	CA670456	wlsu1.pk026.n9 wlsu1 Triticum aestivum cDNA clone wlsu1.pk026.n9 5' end	3,249	6
Ta.6051.1.S1_a_at	CD914245	Os10g0416500 protein n=3 Tax=Oryza sativa RepID=Q9FYR9_ORYSJ	3,239	6
Ta.20549.1.S1_x_at	CA668159	Putative uncharacterized protein Sb04g030310 n=1 Tax=Sorghum bicolor RepID=C5Y093_SORBI	3,227	6
TaAffx.81496.1.S1_at	CA685342	Putative uncharacterized protein Sb05g019490 n=1 Tax=Sorghum bicolor RepID=C5Y384_SORBI	3,220	6
Ta.30336.1.S1_x_at	AB076807	Group2 late embryogenesis abundant protein n=3 Tax=Triticum RepID=Q8LP43_WHEAT	3,214	5
Ta.30860.1.S1_at	CN010964	WHE3878_C08_F16ZS Wheat Fusarium graminearum infected spike cDNA Triticum aestivum	3,203	6
Ta.2709.1.S1_s_at	CK166154	Defensin-like protein 2 n=1 Tax=Triticum aestivum RepID=DEF2_WHEAT	3,188	1
Ta.13784.1.S1_at	AL823038	BLT14.1 protein n=2 Tax=Triticeae RepID=Q40032_HORVU	3,178	5
TaAffx.109191.1.S1_at	BQ168959	WRKY transcription factor n=1 Tax=Triticum aestivum RepID=A3FBG2_WHEAT	3,146	2
TaAffx.110222.1.S1_x_at	CA661932	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays RepID=B6STG1_MAIZE	3,144	5
Ta.22565.1.S1_x_at	BT009372	UDP-glucosyl transferase n=1 Tax=Triticum aestivum RepID=C5HUX8_WHEAT	3,141	3
TaAffx.24475.1.S1_x_at	CA718969	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,123	3
Ta.27279.1.S1_at	BT009316	Os06g0330400 protein n=2 Tax=Oryza sativa RepID=Q69UX2_ORYSJ	3,114	6
TaAffx.108556.1.S1_x_at	CA692789	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B4FS23_MAIZE	3,113	6
Ta.28917.1.S1_at	CK166116	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	3,099	5
TaAffx.84552.1.S1_at	CA634441	wle1n.pk0086.g12 wle1n Triticum aestivum cDNA clone wle1n.pk0086.g12 5' end	3,096	6
TaAffx.65068.1.A1_at	BJ263737	MADS-box transcription factor TaAGL42 n=1 Tax=Triticum aestivum RepID=Q1G167_WHEAT	3,081	2
TaAffx.71225.1.A1_at	BE492103	WHE0551_F06_F06ZE Triticum monococcum vegetative apex cDNA Triticum monococcum	3,073	6
Ta.21646.1.S1_x_at	CA686121	Non-specific lipid-transfer protein n=3 Tax=Oryza sativa RepID=Q75GN2_ORYSJ	3,071	5
Ta.5235.1.S1_x_at	BG606752	Peroxidase n=2 Tax=Triticeae RepID=O49866_HORVU	3,056	4
Ta.21326.1.S1_a_at	AJ613350	Os07g0518100 protein n=3 Tax=Oryza sativa RepID=Q7EZ52_ORYSJ	3,052	6
Ta.10617.1.S1_at	BQ166125	Os01g0108400 protein n=4 Tax=Oryza sativa RepID=Q0JRC6_ORYSJ	3,002	6
Ta.28917.1.S1_x_at	CK166116	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	2,978	5

TaAffx.113315.1.S1_at	CA615901	Iron-phytosiderophore transporter n=1 Tax=Hordeum vulgare RepID=Q2PGC4_HORVU	2,967	5
Ta.25531.2.A1_at	CA680295	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	2,964	5
Ta.6051.3.S1_x_at	CA717933	Os10g0416500 protein n=3 Tax=Oryza sativa RepID=Q9FYR9_ORYSJ	2,956	6
Ta.30921.1.S1_x_at	CN012317	12-oxo-phytodienoic acid reductase n=1 Tax=Zea mays RepID=Q49HE1_MAIZE	2,948	5
Ta.28233.2.S1_a_at	CA695499	Putative uncharacterized protein Sb10g024350 n=1 Tax=Sorghum bicolor RepID=C5Z653_SORBI	2,934	6
Ta.5385.1.S1_at	X85228	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,933	4
Ta.12517.1.S1_at	CK163074	Sugar transport protein 1 n=2 Tax=Zea mays RepID=Q6B4G9_MAIZE	2,900	3
Ta.7022.1.S1_s_at	BJ281221	Phenylalanine ammonia-lyase n=5 Tax=Poaceae RepID=Q7F929_ORYSJ	2,868	1
Ta.18203.1.S1_at	AF031195	Blue copper-binding protein homolog n=1 Tax=Triticum aestivum RepID=Q9ZTU7_WHEAT	2,824	2
TaAffx.109191.1.S1_x_at	BQ168959	WRKY transcription factor n=1 Tax=Triticum aestivum RepID=A3FBG2_WHEAT	2,808	2
TaAffx.114390.1.S1_at	CA593923	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B6SJC9_MAIZE	2,750	6
Ta.13991.1.S1_x_at	CA666889	Putative uncharacterized protein Sb03g043610 n=1 Tax=Sorghum bicolor RepID=C5XG61_SORBI	2,750	6
Ta.24730.2.S1_x_at	BE430562	Protochlorophyllide reductase (Fragment) n=4 Tax=Poaceae RepID=POR_AVESA	2,743	5
TaAffx.16032.1.A1_at	BQ800652	Cell wall invertase n=1 Tax=Lolium perenne RepID=Q2QI10_LOLPR	2,730	5
Ta.28613.1.S1_at	U73213	Cold acclimation protein WCOR726 n=3 Tax=Triticeae RepID=P93610_WHEAT	2,708	5
TaAffx.50125.2.S1_at	CA745672	Cytochrome P450 n=1 Tax=Triticum aestivum RepID=Q9AVM3_WHEAT	2,707	4
TaAffx.85775.1.S1_at	CA621227	Cytochrome P450 monooxygenase CYP71U4v2 n=2 Tax=Hordeum vulgare RepID=Q52PG2_HORVD	2,680	4
TaAffx.137429.1.S1_at	CA610138	Dehydrin 5 (Fragment) n=1 Tax=Hordeum vulgare subsp. spontaneum RepID=Q6V7D2_HORSP	2,645	1
Ta.9332.1.S1_x_at	BQ789066	Os09g0441400 protein n=2 Tax=Oryza sativa RepID=Q69P73_ORYSJ	2,643	6
Ta.5654.1.S1_at	BJ286960	Blue copper-binding protein n=1 Tax=Dasypyrum villosum RepID=A7UHA3_9POAL	2,637	2
Ta.3869.2.S1_at	CA735969	Triticum aestivum cDNA, clone: WT008_I09, cultivar: Chinese Spring	2,636	6
Ta.20549.1.S1_s_at	CA668159	Putative uncharacterized protein Sb04g030310 n=1 Tax=Sorghum bicolor RepID=C5Y093_SORBI	2,621	6
Ta.12434.1.S1_at	CD862952	OSJNBa0086B14.7 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q7XV48_ORYSJ	2,618	6
TaAffx.81921.1.S1_at	CA680100	Os01g0613500 protein n=2 Tax=Oryza sativa RepID=Q9FTI3_ORYSJ	2,617	6
Ta.7022.2.S1_at	BF199967	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,604	1
Ta.3828.3.A1_x_at	BJ254343	Putative glucan endo-1,3-beta-D-glucosidase (Fragment) n=1 Tax=Triticum aestivum	2,596	3
TaAffx.15958.1.S1_at	BQ801634	Os01g0914100 protein n=2 Tax=Oryza sativa RepID=Q8S077_ORYSJ	2,595	6
Ta.25077.1.A1_at	BQ161103	Ice recrystallization inhibition protein 2 n=1 Tax=Triticum aestivum RepID=Q56B89_WHEAT	2,580	2
TaAffx.120890.1.S1_x_at	CA688266	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	2,575	5
Ta.7022.2.S1_x_at	BF199967	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,569	1
Ta.24106.1.S1_x_at	CA665159	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,569	4
Ta.11016.1.S1_at	BQ168385	Calmodulin-like protein n=1 Tax=Zea mays RepID=B6TXW9_MAIZE	2,565	5
Ta.13956.1.S1_at	BJ287532	cDNA library, Wh_r Triticum aestivum cDNA clone whr20i23 3'	2,559	6
Ta.10581.1.A1_at	CK156211	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf14j06, mRNA sequence	2,552	6
TaAffx.24109.1.A1_at	CK216113	Os03g0400200 protein n=4 Tax=Oryza sativa RepID=Q10K20_ORYSJ	2,547	6
Ta.231.1.S1_at	AF079526	PR17c n=2 Tax=Triticeae RepID=A7YA60_HORVD	2,547	6
TaAffx.124475.1.A1_at	CK212125	BLT14.2 protein n=2 Tax=Hordeum vulgare RepID=Q40033_HORVU	2,528	6

TaAffx.115935.1.S1_x_at	AY596267	Polyphenol oxidase n=2 Tax=Triticum aestivum RepID=C0SPI5_WHEAT	2,515	4
Ta.21556.1.S1_at	CA684533	Protein WIR1B n=1 Tax=Triticum aestivum RepID=WIR1B_WHEAT	2,505	1
Ta.25026.1.S1_at	BQ804965	Dehydrin n=1 Tax=Triticum turgidum subsp. durum RepID=Q5CAQ2_TRITU	2,504	1
Ta.11397.1.A1_at	BQ170011	Putative uncharacterized protein Sb02g035400 n=1 Tax=Sorghum bicolor RepID=C5XAR6_SORBI	2,498	6
TaAffx.55592.1.S1_at	CA663917	Potassium transporter 5 n=1 Tax=Oryza sativa Japonica Group RepID=HAK5_ORYSJ	2,481	5
Ta.21768.1.S1_x_at	CA701727	Ice recrystallization inhibition protein 7 n=1 Tax=Deschampsia antarctica RepID=C0L704_DESAN	2,475	2
Ta.21272.1.S1_at	CA668970	Cell division AAA ATPase family protein n=10 Tax=Triticeae RepID=B6Z264_WHEAT	2,470	5
Ta.223.1.S1_at	AF112965	Beta-1,3-glucanase n=1 Tax=Triticum aestivum RepID=Q9XEN5_WHEAT	2,464	3
Ta.30913.1.A1_at	CN012655	Os09g0454600 protein n=3 Tax=Oryza sativa RepID=Q0J198_ORYSJ	2,461	6
Ta.22628.1.S1_x_at	CA690208	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B6SP49_MAIZE	2,453	6
TaAffx.65294.1.A1_at	BJ252171	Chloroplast lipocalin n=2 Tax=Triticeae RepID=Q38JB3_WHEAT	2,437	4
Ta.22628.1.S1_at	CA690208	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B6SP49_MAIZE	2,436	6
Ta.8228.1.S1_at	BQ161624	Agmatine coumaroyltransferase n=3 Tax=Hordeum vulgare RepID=A9ZPJ6_HORVU	2,432	5
Ta.3703.2.S1_s_at	BJ246711	Cytochrome P450 CYP709E4 n=2 Tax=Zea mays RepID=B6TF97_MAIZE	2,431	4
Ta.16472.1.S1_s_at	CA606887	Pathogenesis-related protein n=1 Tax=Hordeum vulgare RepID=P93181_HORVU	2,418	1
TaAffx.109085.1.S1_at	CA680382	wlm24.pk0006.g11 wlm24 Triticum aestivum cDNA clone wlm24.pk0006.g11 5' end	2,417	6
TaAffx.99316.1.A1_at	CK207671	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9GE50_ORYSJ	2,412	6
Ta.20570.1.A1_at	CA741536	1-aminocyclopropane-1-carboxylate oxidase (Fragment) n=2 Tax=Andropogoneae RepID=O81607_SORBI	2,411	4
Ta.20197.1.S1_at	CA674805	Putative uncharacterized protein (Fragment) n=2 Tax=Papilionoideae RepID=B7FFL2_MEDTR	2,410	6
TaAffx.34169.1.S1_at	BJ287371	Putative uncharacterized protein n=2 Tax=Oryza sativa RepID=B9FBJ1_ORYSJ	2,410	6
Ta.11421.1.A1_at	BQ170075	Os01g0266500 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q9SDD7_ORYSJ	2,401	6
Ta.23366.3.A1_x_at	BQ161967	Class III peroxidase 62 n=2 Tax=Oryza sativa Japonica Group RepID=Q5U1N1_ORYSJ	2,400	4
Ta.351.2.S1_x_at	CA673444	Cold acclimation induced protein 2-1 n=1 Tax=Triticum aestivum RepID=Q4KXE0_WHEAT	2,400	5
Ta.23397.1.S1_x_at	BJ292777	Glycine-rich cell wall structural protein n=1 Tax=Hordeum vulgare RepID=GRP1_HORVU	2,390	5
Ta.25487.1.S1_at	CD373987	Putative uncharacterized protein Sb02g035450 n=1 Tax=Sorghum bicolor RepID=C5XAS1_SORBI	2,385	6
Ta.23397.2.S1_x_at	CA718270	Pherophorin-dz1 protein n=1 Tax=Volvox carteri f. nagariensis RepID=Q8L685_VOLCA	2,384	5
Ta.28659.1.S1_x_at	CA670381	Putative protease inhibitor n=1 Tax=Hordeum vulgare RepID=Q96465_HORVU	2,384	5
Ta.25539.1.S1_at	CA678031	BLT14.1 protein n=2 Tax=Triticeae RepID=Q40032_HORVU	2,362	6
Ta.18720.3.S1_x_at	BJ295779	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	2,357	5
Ta.4696.1.S1_at	BJ275186	Os01g0366300 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q5Z8B7_ORYSJ	2,353	6
Ta.21650.1.A1_at	CA667760	NBS-LRR resistance-like protein n=1 Tax=Hordeum vulgare RepID=A9UKM2_HORVU	2,343	5
Ta.12663.1.S1_at	CK197682	Ice recrystallization inhibition protein 1 n=1 Tax=Triticum aestivum RepID=Q56B90_WHEAT	2,334	2
TaAffx.51261.1.S1_at	CA728497	Os01g0793900 protein n=2 Tax=Oryza sativa RepID=Q8S1K8_ORYSJ	2,329	6
TaAffx.128418.43.S1_at	BJ252866	Endochitinase n=5 Tax=Pooideae RepID=Q41539_WHEAT	2,324	1
TaAffx.80306.1.S1_at	CA698528	1-aminocyclopropane-1-carboxylate oxidase (Fragment) n=1 Tax=Triticum monococcum	2,322	4
Ta.21281.1.S1_at	BQ162027	PDR-type ABC transporter n=2 Tax=Triticum aestivum RepID=B9UYP3_WHEAT	2,320	5

Ta.24254.1.S1_a_at	CA616263	Plastid omega-3 fatty acid desaturase n=2 Tax=Oryza sativa Japonica Group RepID=Q2HWS9_ORYSJ	2,319	5
TaAffx.20635.1.S1_at	CD895869	Sugar transporter family protein, expressed n=3 Tax=Oryza sativa RepID=Q10QG4_ORYSJ	2,319	3
Ta.28659.3.S1_x_at	CA689419	Putative protease inhibitor n=1 Tax=Hordeum vulgare RepID=Q96465_HORVU	2,317	1
Ta.19786.1.A1_at	CA658969	Putative uncharacterized protein Sb01g016640 n=1 Tax=Sorghum bicolor RepID=C5WV89_SORBI	2,294	6
Ta.22678.1.A1_a_at	CK214868	Chitinase 1 n=2 Tax=Andropogoneae RepID=B4FBN8_MAIZE	2,294	1
Ta.25754.1.A1_at	CD373766	Putative uncharacterized protein Sb03g045850 n=1 Tax=Sorghum bicolor RepID=C5XHV9_SORBI	2,291	6
Ta.23366.3.A1_at	BQ161967	Class III peroxidase 62 n=2 Tax=Oryza sativa Japonica Group RepID=Q5U1N1_ORYSJ	2,290	4
Ta.3162.1.S1_at	BJ215513	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FSW5_ORYSJ	2,286	6
Ta.29496.1.S1_x_at	CK195383	Peroxidase 12 n=2 Tax=Zea mays RepID=B6THG0_MAIZE	2,281	4
Ta.21505.1.S1_at	CK213957	Root peroxidase n=1 Tax=Triticum aestivum RepID=B4F6E8_WHEAT	2,276	4
Ta.25542.1.S1_at	CD896341	Os02g0712700 protein n=3 Tax=Oryza sativa RepID=Q0DY65_ORYSJ	2,273	6
Ta.18720.1.S1_a_at	BJ288588	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	2,267	5
Ta.8399.2.S1_at	CA694080	Putative uncharacterized protein Sb03g028700 n=1 Tax=Sorghum bicolor RepID=C5XEF4_SORBI	2,266	6
TaAffx.45277.1.S1_x_at	BJ231180	Phenylalanine ammonia-lyase n=1 Tax=Triticum aestivum RepID=PALY_WHEAT	2,263	1
Ta.8512.1.S1_at	BQ162001	Os07g0631700 protein n=2 Tax=Oryza sativa RepID=Q8LHN5_ORYSJ	2,254	6
Ta.2278.2.S1_a_at	CK196331	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	2,254	1
Ta.6187.1.S1_at	BQ167028	Glycine-rich cell wall structural protein n=2 Tax=Zea mays RepID=B6ST85_MAIZE	2,249	5
TaAffx.82108.1.S1_x_at	CA677464	Os01g0115700 n=1 Tax=Oryza sativa Japonica Group RepID=UPI0000DD891C	2,249	6
Ta.351.1.S1_at	CA669389	Cold acclimation induced protein 2-1 n=1 Tax=Triticum aestivum RepID=Q4KXE0_WHEAT	2,247	5
Ta.24254.2.S1_at	BQ800827	Os03g0290300 protein (Fragment) n=1 Tax=Oryza sativa Japonica Group RepID=Q0DSS9_ORYSJ	2,246	6
TaAffx.98930.1.A1_at	CK214676	CBF12 n=8 Tax=Triticeae RepID=B1NSN2_TRIMO	2,242	2
Ta.13232.2.S1_at	BQ166297	WHE0840_F03_L06ZT Wheat vernalized crown cDNA library Triticum aestivum	2,240	6
Ta.8614.1.S1_at	BQ838257	WRKY45 transcription factor n=1 Tax=Triticum aestivum RepID=A3RG93_WHEAT	2,237	2
Ta.2278.3.S1_x_at	CD490414	Chitinase II n=1 Tax=Triticum aestivum RepID=Q9XEN3_WHEAT	2,234	1
TaAffx.131248.2.S1_at	CA670789	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf2f01, mRNA sequence	2,223	6
TaAffx.83027.1.S1_at	CA662924	Os05g0135100 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q0DKY8_ORYSJ	2,221	6
Ta.4921.1.S1_at	BE438217	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FAG9_ORYSJ	2,216	6
Ta.15129.1.S1_at	CA690804	wlm96.pk052.p8 wlm96 Triticum aestivum cDNA clone wlm96.pk052.p8 5' end	2,215	6
TaAffx.28302.4.S1_at	CA662104	wlmk1.pk0015.d5 wlmk1 Triticum aestivum cDNA clone wlmk1.pk0015.d5 5' end	2,208	6
Ta.4725.1.S1_at	CK195830	WRKY14 transcription factor n=1 Tax=Triticum aestivum RepID=B3GAU5_WHEAT	2,207	2
Ta.27389.2.S1_x_at	BJ297034	Defensin-like protein 2 n=1 Tax=Triticum aestivum RepID=DEF2_WHEAT	2,202	1
Ta.21307.1.S1_x_at	CK199589	Peroxidase 12 n=2 Tax=Zea mays RepID=B6THG0_MAIZE	2,186	4
Ta.24832.1.S1_s_at	CA668285	Os08g0127100 protein n=6 Tax=Poaceae RepID=Q6ZK52_ORYSJ	2,182	6
TaAffx.16900.1.A1_at	CK215179	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=Q7X669_ORYSJ	2,180	6
Ta.22625.1.A1_s_at	CA666706	Os02g0634700 protein n=2 Tax=Oryza sativa RepID=Q6H7I7_ORYSJ	2,179	6
Ta.30495.1.A1_s_at	CK214893	CBFIVc-14.1 n=2 Tax=Triticum RepID=A0MPL5_WHEAT	2,176	2
Ta.303.3.S1_x_at	AJ610447	Glutathione S-transferase 2 n=2 Tax=Triticum aestivum	2,175	4

		RepID=GSTF2_WHEAT		
TaAffx.113701.1.S1_s_at	CA606782	Pleiotropic drug resistance protein 4 n=3 Tax=Oryza sativa RepID=PDR4_ORYSJ	2,172	5
Ta.169.1.S1_x_at	CA652856	Germin-like 12 n=7 Tax=Triticeae RepID=Q43487_HORVU	2,170	5
Ta.21035.1.S1_at	CA614540	Putative uncharacterized protein Sb03g006590 n=1 Tax=Sorghum bicolor RepID=C5XQN8_SORBI	2,166	6
TaAffx.131248.2.S1_s_at	CA670789	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf2f01, mRNA sequence	2,165	6
TaAffx.21249.1.S1_at	CA706624	Os02g0720600 protein n=2 Tax=Oryza sativa RepID=Q6Z674_ORYSJ	2,162	6
TaAffx.3462.1.S1_at	CK215505	Early salt stress and cold acclimation-induced protein 2-1 n=1 Tax=Lophopyrum elongatum	2,161	5
Ta.12671.1.S1_a_at	CK194385	Putative uncharacterized protein Sb04g024020 n=1 Tax=Sorghum bicolor RepID=C5XVR6_SORBI	2,160	6
TaAffx.5899.1.S1_at	CA696250	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2YM74_ORYSI	2,156	6
Ta.27506.1.S1_at	AY428038	Ammonium transporter AMT2.1 n=1 Tax=Triticum aestivum RepID=Q6T8L6_WHEAT	2,155	5
TaAffx.108908.1.S1_x_at	CA684616	Os03g0663500 protein (Fragment) n=3 Tax=Oryza sativa RepID=Q0DPU1_ORYSJ	2,153	6
Ta.27455.1.S1_at	CA644954	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	2,152	6
Ta.2793.1.S1_at	AB055077	Multidrug resistance protein 1 homolog n=1 Tax=Triticum aestivum RepID=Q8RVT7_WHEAT	2,151	5
TaAffx.77715.1.S1_at	CA744342	S-domain receptor-like protein kinase n=1 Tax=Oryza granulata RepID=B9V0L1_9ORYZ	2,151	5
Ta.21250.1.S1_at	BJ273029	MADS-box transcription factor TaAGL23 n=4 Tax=Triticeae RepID=Q1G185_WHEAT	2,144	2
Ta.12441.1.A1_at	BQ172342	Putative uncharacterized protein n=3 Tax=Zea mays RepID=C4JAI5_MAIZE	2,137	6
Ta.8076.1.S1_at	CA684491	Os02g0634700 protein n=2 Tax=Oryza sativa RepID=Q6H7I7_ORYSJ	2,135	6
Ta.28224.1.S1_x_at	CK193135	Os01g0384800 protein n=2 Tax=Oryza sativa RepID=Q5VNY3_ORYSJ	2,134	6
Ta.6990.1.S1_at	CA611113	Pleiotropic drug resistance protein 4 n=3 Tax=Oryza sativa RepID=PDR4_ORYSJ	2,133	5
TaAffx.21249.1.S1_x_at	CA706624	Os02g0720600 protein n=2 Tax=Oryza sativa RepID=Q6Z674_ORYSJ	2,133	6
TaAffx.82108.1.S1_at	CA677464	Os01g0115700 n=1 Tax=Oryza sativa Japonica Group RepID=UPI0000DD891C	2,131	6
Ta.5824.2.S1_x_at	AL830800	Os02g0740600 protein n=2 Tax=Oryza sativa RepID=Q6Z7S9_ORYSJ	2,124	6
TaAffx.54530.1.S1_at	CA633759	Os04g0103500 protein n=2 Tax=Oryza sativa RepID=Q7XMR2_ORYSJ	2,122	6
TaAffx.81369.1.S1_at	CA686927	Receptor-like protein kinase n=2 Tax=Oryza sativa RepID=Q9M575_ORYSA	2,099	5
TaAffx.80313.1.S1_at	CA698434	Putative WRKY5 protein (Fragment) n=1 Tax=Hordeum vulgare RepID=Q5W1F7_HORVU	2,094	2
Ta.27258.1.S1_at	BT009386	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays	2,091	5
Ta.8323.1.A1_at	BQ161741	Os02g0515200 protein n=2 Tax=Oryza sativa RepID=Q67UP1_ORYSJ	2,090	6
TaAffx.83591.1.S1_at	CA655732	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8B433_ORYSI	2,087	6
Ta.24710.1.S1_at	CA678411	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,087	4
Ta.2278.2.S1_x_at	CK196331	Chitinase IV n=1 Tax=Triticum aestivum RepID=Q9XEN6_WHEAT	2,086	1
Ta.14837.1.S1_at	CA682925	Calreticulin, putative n=1 Tax=Ricinus communis RepID=B9RM48_RICCO	2,075	5
TaAffx.8335.1.S1_at	CA627027	wl1n.pk151.c8 wl1n Triticum aestivum cDNA clone wl1n.pk151.c8 5' end	2,068	6
Ta.25845.1.S1_at	CD453621	Putative uncharacterized protein Sb09g003460 n=1 Tax=Sorghum bicolor RepID=C5Z001_SORBI	2,052	6
Ta.10549.2.A1_at	BQ165963	Alternative oxidase n=3 Tax=Oryza sativa RepID=O82807_ORYSJ	2,050	4
TaAffx.15674.1.A1_at	CD490328	WHE2494_H02_P04ZT Triticum monococcum DV92 early reproductive apex	2,044	6
Ta.3154.1.S1_at	BJ223797	Sorghum bicolor hypothetical protein (SORBIDRAFT_0019s004610) mRNA	2,043	5

Ta.24723.1.S1_x_at	CA667447	PSBGer1 protein n=1 Tax=Triticum aestivum RepID=P93598_WHEAT	2,043	5
Ta.4831.1.S1_at	AJ611741	Putative uncharacterized protein Sb06g032110 n=1 Tax=Sorghum bicolor RepID=C5YA11_SORBI	2,042	6
Ta.1830.2.S1_x_at	CA683961	Putative uncharacterized protein Sb01g030980 n=1 Tax=Sorghum bicolor RepID=C5WUJ5_SORBI	2,037	6
Ta.21711.1.S1_at	CD883645	Putative uncharacterized protein Sb07g020050 n=1 Tax=Sorghum bicolor RepID=C5YKT1_SORBI	2,028	6
TaAffx.56501.1.S1_at	BU099852	Sorghum bicolor hypothetical protein (SORBIDRAFT_0019s004610) mRNA, complete cds	2,026	5
TaAffx.128683.1.S1_x_at	CA684810	Cell division AAA ATPase family protein n=10 Tax=Triticeae RepID=B6Z264_WHEAT	2,022	5
TaAffx.31754.1.S1_at	CA608501	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FXP2_ORYSJ	2,020	6
Ta.3828.3.A1_a_at	BJ254343	Putative glucan endo-1,3-beta-D-glucosidase (Fragment) n=1 Tax=Triticum aestivum	2,018	3
TaAffx.70641.1.S1_at	BQ802487	WHE2826_E02_104ZS Triticum monococcum vernalized apex	2,018	6
Ta.19609.1.S1_at	AJ615628	Cytochrome P450 n=1 Tax=Triticum aestivum RepID=Q2V065_WHEAT	2,015	4
Ta.26236.1.A1_at	CD453043	Abhydrolase domain-containing protein 5 n=2 Tax=Andropogoneae RepID=B4FAQ7_MAIZE	2,005	5
Ta.28827.1.S1_at	BQ800700	Histone H1 n=2 Tax=Triticum aestivum RepID=O65795_WHEAT	2,004	5
TaAffx.4290.1.A1_at	CA681239	wlm24.pk0014.c7 wlm24 Triticum aestivum cDNA clone wlm24.pk0014.c7 5' end.	2,001	6
TaAffx.130076.1.S1_at	BQ168759	Os05g0550600 protein n=2 Tax=Oryza sativa RepID=Q6L4G9_ORYSJ	-2,002	6
Ta.1282.4.S1_at	CA635994	Non-specific lipid-transfer protein n=3 Tax=Triticeae RepID=Q42848_HORVU	-2,017	5
TaAffx.95414.1.S1_at	BQ803340	Branched-chain-amino-acid aminotransferase n=1 Tax=Hordeum vulgare	-2,020	5
TaAffx.18447.3.S1_s_at	CA737476	Putative uncharacterized protein Sb08g020600 n=1 Tax=Sorghum bicolor	-2,022	6
Ta.22101.1.A1_at	CA721955	Chlorophyll a-b binding protein 22L, chloroplastic n=14 Tax=Solanaceae	-2,022	2
Ta.2926.1.A1_at	BJ207389	Triticum aestivum cDNA, clone: WT009_B01, cultivar: Chinese Spring	-2,022	6
Ta.2969.1.A1_at	CK165153	Expansin EXPB2 n=1 Tax=Triticum aestivum RepID=Q6QFA2_WHEAT	-2,024	5
Ta.28728.1.S1_at	AF139815	Aquaporin PIP2-4 n=9 Tax=Poaceae RepID=PIP24_MAIZE	-2,035	6
Ta.8640.1.S1_a_at	BQ807183	Os07g0169600 protein n=2 Tax=Oryza sativa RepID=Q69LD9_ORYSJ	-2,037	6
Ta.6558.1.S1_x_at	CK208447	Os03g0787200 protein n=2 Tax=Oryza sativa RepID=Q6F3B1_ORYSJ	-2,042	6
Ta.28847.1.S1_a_at	CA740446	Os01g0795100 protein n=2 Tax=Oryza sativa Japonica Group RepID=Q8S1I0_ORYSJ	-2,056	6
Ta.20938.2.A1_x_at	CA624118	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,066	6
TaAffx.113526.2.S1_s_at	CA723504	Proline-rich protein n=2 Tax=Triticum aestivum RepID=C7EZF0_WHEAT	-2,081	5
TaAffx.63920.1.A1_x_at	BQ162587	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf149n21, mRNA sequence	-2,084	6
Ta.3857.1.A1_at	CA698230	Os08g0137400 protein n=3 Tax=Oryza sativa RepID=Q0J845_ORYSJ	-2,090	6
Ta.6374.3.S1_a_at	BJ300310	Squamosa promoter-binding-like protein 13 n=3 Tax=Oryza sativa RepID=SPL13_ORYSJ	-2,092	2
Ta.28728.1.S1_x_at	AF139815	Aquaporin PIP2-4 n=9 Tax=Poaceae RepID=PIP24_MAIZE	-2,093	5
TaAffx.8097.1.S1_x_at	CA636056	wle1n.pk0106.b2 wle1n Triticum aestivum cDNA clone wle1n.pk0106.b2 5' end	-2,125	6
Ta.28422.1.A1_s_at	BJ317142	Os03g0724600 protein (Fragment) n=3 Tax=Oryza sativa RepID=Q0DP01_ORYSJ	-2,141	6
TaAffx.8804.2.S1_at	BE217049	Probable aquaporin PIP2-7 n=3 Tax=Oryza sativa RepID=PIP27_ORYSJ	-2,144	5
Ta.7724.3.S1_at	CA631315	Putative uncharacterized protein Sb09g001060 n=1 Tax=Sorghum bicolor	-2,145	6
Ta.8710.1.A1_at	BQ162259	Triticum aestivum cDNA, clone: WT012_L15, cultivar: Chinese Spring	-2,167	6
Ta.6934.1.A1_x_at	CK209084	UPI0000D8C4F1 related cluster n=1 Tax=Danio rerio RepID=UPI0000D8C4F1	-2,170	6
Ta.30144.1.A1_x_at	BQ166180	Proline-rich protein n=1 Tax=Sorghum bicolor RepID=A7KH48_SORBI	-2,190	5

TaAffx.15847.3.S1_at	BQ802374	Xyloglucan endotransglycosylase (XET) n=1 Tax=Hordeum vulgare RepID=P93671_HORVU	-2,200	3
Ta.20540.3.S1_x_at	BJ318550	Putative uncharacterized protein Sb01g004270 n=2 Tax=Andropogoneae	-2,210	6
Ta.3.1.S1_at	BJ265463	Beta-amylase n=2 Tax=Triticeae RepID=AMYB_WHEAT	-2,226	5
Ta.20540.3.S1_at	BJ318550	Putative uncharacterized protein Sb01g004270 n=2 Tax=Andropogoneae	-2,229	6
Ta.11684.1.A1_at	BQ170589	Os03g0234900 protein n=2 Tax=Oryza sativa RepID=Q5U1Q4_ORYSJ	-2,240	6
Ta.20938.1.A1_at	BQ169082	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,241	6
Ta.12820.1.S1_at	CK215415	Defensin n=1 Tax=Triticum turgidum subsp. durum RepID=C9E1C6_TRITU	-2,245	1
TaAffx.43693.1.S1_at	BF199968	Xet3 protein n=1 Tax=Festuca pratensis RepID=Q949H9_FESPR	-2,268	5
TaAffx.71208.1.S1_at	BE492167	Amidase n=2 Tax=Zea mays RepID=B6TMI1_MAIZE	-2,269	5
Ta.20938.1.A1_a_at	BQ169082	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,271	6
TaAffx.16068.1.S1_at	BQ168935	Oryza sativa Japonica Group cDNA clone:002-112-B01, full insert sequence	-2,273	6
Ta.7724.3.S1_x_at	CA631315	Putative uncharacterized protein Sb09g001060 n=1 Tax=Sorghum bicolor	-2,281	6
TaAffx.8804.1.S1_s_at	CK164001	Probable aquaporin PIP2-7 n=3 Tax=Oryza sativa RepID=PIP27_ORYSJ	-2,287	5
Ta.13160.1.S1_at	CK195065	Stem-specific protein n=4 Tax=Andropogoneae RepID=Q5U7K5_9POAL	-2,289	5
Ta.14454.1.S1_s_at	BJ215196	Os09g0433800 protein n=3 Tax=Oryza sativa RepID=Q69PH9_ORYSJ	-2,293	6
Ta.1600.1.A1_at	CK164799	Nodulin-like protein 5NG4 n=3 Tax=Andropogoneae RepID=B6TLA9_MAIZE	-2,301	5
Ta.10400.1.S1_at	BJ251360	Cortical cell-delineating protein n=7 Tax=Zea mays RepID=B6SIN6_MAIZE	-2,324	5
TaAffx.632.1.A1_at	CD490405	Hordeum vulgare ENOD40-like protein mRNA, complete cds	-2,325	5
Ta.5563.1.S1_at	CA643873	Putative uncharacterized protein OSJNBa0027N19.12 n=2 Tax=Oryza sativa	-2,341	6
Ta.9000.1.S1_at	CD453515	Acid beta-fructofuranosidase n=1 Tax=Triticum aestivum RepID=Q575T1_WHEAT	-2,378	3
Ta.6934.1.A1_a_at	CK209084	UPI0000D8C4F1 related cluster n=1 Tax=Danio rerio RepID=UPI0000D8C4F1	-2,385	6
TaAffx.36760.1.S1_at	BJ315664	CEN-like protein 2, putative, expressed n=13 Tax=Poaceae RepID=Q53Q71_ORYSJ	-2,392	5
Ta.2636.1.S1_x_at	CA605844	Putative uncharacterized protein Sb09g024850 n=2 Tax=Andropogoneae	-2,397	6
Ta.28847.1.S1_at	CA740446	Os01g0795100 protein n=2 Tax=Oryza sativa Japonica Group RepID=Q8S1I0_ORYSJ	-2,416	6
Ta.26213.1.S1_at	CD452838	Putative uncharacterized protein Sb06g022610 n=2 Tax=Andropogoneae	-2,418	6
Ta.23219.1.A1_x_at	CA737258	wpi2s.pk001.n10 wpi2s Triticum aestivum cDNA clone wpi2s.pk001.n10 5' end	-2,422	6
Ta.28133.1.A1_s_at	CA636835	Dirigent-like protein, expressed n=2 Tax=Oryza sativa RepID=Q2R0I1_ORYSJ	-2,438	1
Ta.22954.3.S1_at	CA632583	Triticum aestivum cultivar Renan clone BAC 930H14, complete sequence	-2,441	6
TaAffx.84069.1.S1_at	CA645540	Os03g0859100 protein n=2 Tax=Oryza sativa RepID=Q84M86_ORYSJ	-2,501	6
Ta.7963.2.S1_x_at	CK215257	Dirigent-like protein, expressed n=2 Tax=Oryza sativa RepID=Q2R0I1_ORYSJ	-2,584	1
Ta.346.1.A1_at	BJ254518	Cycloartenol synthase, putative, expressed n=1 Tax=Oryza sativa Japonica Group	-2,616	5
Ta.7388.2.S1_x_at	BU672305	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-2,636	1
TaAffx.16936.1.S1_at	CK216481	Fasciclin-like protein FLA16 n=2 Tax=Triticum aestivum RepID=Q06I90_WHEAT	-2,665	5
Ta.24294.1.A1_at	BQ172428	Triticum aestivum cDNA clone WHE2064_H07_O14.	-2,693	6
Ta.1037.1.S1_at	CA638909	Os01g0731100 protein n=4 Tax=Oryza sativa RepID=Q94EA4_ORYSJ	-2,700	6
TaAffx.143995.17.A1_at	AY188331	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-2,714	2
TaAffx.16307.1.S1_at	BQ800749	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf143f08, mRNA sequence	-2,716	6

Ta.20696.3.S1_x_at	CA598744	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-2,767	5
Ta.3304.1.S1_at	BJ243222	Putative uncharacterized protein Sb05g001550 n=1 Tax=Sorghum bicolor	-2,804	6
Ta.4455.1.A1_at	BJ253690	Os01g0842500 protein n=2 Tax=Oryza sativa RepID=Q8S2A8_ORYSJ	-2,810	6
Ta.9216.1.A1_a_at	BQ165936	Homeobox-leucine zipper protein HOX12 n=3 Tax=Oryza sativa RepID=HOX12_ORYSJ	-2,813	2
Ta.6965.1.S1_at	BJ313225	Wh_yf Triticum aestivum cDNA clone whyf4e06 5'.	-2,845	6
Ta.3813.1.A1_at	BQ238416	Putative uncharacterized protein Sb05g026610 n=1 Tax=Sorghum bicolor	-3,158	6
Ta.9058.1.S1_at	CA642666	Acid beta-fructofuranosidase n=1 Tax=Triticum aestivum RepID=Q575T1_WHEAT	-3,184	3
Ta.29640.1.S1_x_at	CK205489	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B7ZYD6_MAIZE	-3,213	6
Ta.30640.1.S1_at	CD861747	Triticum aestivum flowering locus T mRNA, complete cds or VRN3	-3,250	2
TaAffx.410.1.S1_s_at	CA646394	Sucrose synthase metabolism (Fragment) n=3 Tax=Poaceae RepID=A6MZV1_ORYSI	-3,263	3
Ta.438.2.S1_x_at	CK212622	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,276	5
Ta.22954.1.S1_at	CA673824	Putative thionin Osthi1 n=2 Tax=Oryza sativa RepID=Q5Z4W6_ORYSJ	-3,384	5
Ta.7108.1.S1_at	CF134173	Ice recrystallization inhibition protein 2 n=1 Tax=Triticum aestivum	-3,427	2
Ta.438.1.S1_x_at	BG908651	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,451	5
Ta.246.1.S1_at	AF224499	Homeobox protein KNOX3 n=3 Tax=Triticeae RepID=KNOX3_HORVU	-3,477	2
Ta.9216.1.A1_x_at	BQ165936	Homeobox-leucine zipper protein HOX12 n=3 Tax=Oryza sativa RepID=HOX12_ORYSJ	-3,497	2
Ta.22954.2.S1_x_at	CA675346	gene="ACT-1" actin [Triticum aestivum] protein_id="AAW78915.1	-3,497	5
Ta.10151.1.S1_at	BJ234600	Putative uncharacterized protein Sb09g016470 n=2 Tax=Sorghum bicolor	-3,503	6
TaAffx.410.1.S1_at	CA646394	Sucrose synthase metabolism (Fragment) n=3 Tax=Poaceae RepID=A6MZV1_ORYSI	-3,519	3
Ta.556.1.S1_x_at	CA671780	Os05g0153300 protein n=3 Tax=Oryza sativa RepID=Q65XP3_ORYSJ	-3,531	6
Ta.438.1.S1_a_at	BG908651	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,696	5
Ta.22954.1.S1_a_at	CA673824	Putative thionin Osthi1 n=2 Tax=Oryza sativa RepID=Q5Z4W6_ORYSJ	-3,839	5
Ta.556.1.S1_at	CA671780	Os05g0153300 protein n=3 Tax=Oryza sativa RepID=Q65XP3_ORYSJ	-3,849	6
Ta.28480.1.S1_s_at	CD452788	Os03g0111200 protein n=3 Tax=Oryza sativa RepID=Q10SU0_ORYSJ	-4,001	6
TaAffx.98064.1.A1_at	BQ168859	Ice recrystallization inhibition protein 3 n=1 Tax=Deschampsia antarctica	-4,022	2
Ta.11506.1.S1_a_at	CK215703	Os07g0617500 protein n=2 Tax=Oryza sativa RepID=Q8GS08_ORYSJ	-4,843	6
Ta.28186.2.A1_a_at	CA671332	Voltage-dependent outwardly rectifying plasma membrane K+ channel KCO1/TPK1 n=4	-5,131	5
Ta.3583.1.A1_at	BJ219585	MADS-box protein 8 n=4 Tax=Pooideae RepID=Q9LEI0_HORVU	-5,963	2
Ta.20696.3.S1_s_at	CA598744	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-6,406	5
Ta.7388.1.S1_at	BJ320233	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-7,187	1
Ta.7388.2.S1_a_at	BU672305	Jasmonate-induced protein n=2 Tax=Triticum aestivum RepID=A7LM74_WHEAT	-7,230	1
TaAffx.36593.1.S1_at	BQ166396	Amylase inhibitor-like protein n=3 Tax=Triticum RepID=A9UID9_WHEAT	-8,802	5
TaAffx.85922.1.S1_x_at	CA618396	Putative uncharacterized protein (Fragment) n=5 Tax=Triticeae RepID=Q9M540_AGRCR	-11,074	6
TaAffx.116496.1.S1_at	CA693971	Phytochrome C n=10 Tax=Triticeae RepID=Q2I714_HORVD	-14,526	5
Ta.21379.1.S1_s_at	CA674527	Voltage-dependent outwardly rectifying plasma membrane K+ channel KCO1/TPK1 n=4	-15,607	5
Ta.28005.1.A1_at	CD862101	Phytochrome C (Fragment) n=1 Tax=Hordeum vulgare RepID=Q945T7_HORVU	-16,267	5
TaAffx.120063.2.S1_s_at	BJ245749	MADS-box transcription factor 18 n=4 Tax=Oryza sativa RepID=MAD18_ORYSJ	-37,146	2

Ta.30607.1.A1_at	BJ264278	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-60,556	2
TaAffx.120063.1.S1_at	CF134093	MADS-box transcription factor 18 n=4 Tax=Oryza sativa RepID=MAD18_ORYSJ	-64,352	2
Ta.6793.1.A1_at	CD492136	MADS2 n=3 Tax=Triticeae RepID=A5X498_WHEAT	-105,103	2
TaAffx.143995.17.S1_s_at	AY188331	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-108,744	2
Ta.7832.1.S1_at	CA646083	Putative uncharacterized protein Sb01g007930 n=2 Tax=Poaceae RepID=C5X0B2_SORBI	-114,258	6
TaAffx.85922.1.S1_s_at	CA618396	Putative uncharacterized protein Sb01g007930 n=2 Tax=Poaceae RepID=C5X0B2_SORBI	-114,866	6
Ta.29481.1.S1_at	CK194207	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-169,064	5

Legend: According to Affymetrix Gene Chip® wheat genome array of the 368 Probe sets IDs diferentially regulated complemented with BLAST results showing the Genbank accession number, UniProt or NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$ and the classes of the genes.

Table S5.2 *mvp* wheat plant class 2 (Transcription factors) regulated genes identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
Ta.26917.1.S1_at	CD452828	MADS-box transcription factor TaAGL41 n=2 Tax=Triticum aestivum RepID=Q1G168_WHEAT	4,600
TaAffx.122374.1.A1_at	BE517594	CBFIVa-2.2 (Fragment) n=1 Tax=Triticum aestivum RepID=A0MPK8_WHEAT	3,525
TaAffx.109191.1.S1_at	BQ168959	WRKY transcription factor n=1 Tax=Triticum aestivum RepID=A3FBG2_WHEAT	3,146
TaAffx.65068.1.A1_at	BJ263737	MADS-box transcription factor TaAGL42 n=1 Tax=Triticum aestivum RepID=Q1G167_WHEAT	3,081
Ta.18203.1.S1_at	AF031195	Blue copper-binding protein homolog n=1 Tax=Triticum aestivum RepID=Q9ZTU7_WHEAT	2,824
TaAffx.109191.1.S1_x_at	BQ168959	WRKY transcription factor n=1 Tax=Triticum aestivum RepID=A3FBG2_WHEAT	2,808
Ta.5654.1.S1_at	BJ286960	Blue copper-binding protein n=1 Tax=Dasypyrum villosum RepID=A7UHA3_9POAL	2,637
TaAffx.98930.1.A1_at	CK214676	CBF12 n=8 Tax=Triticeae RepID=B1NSN2_TRIMO	2,242
Ta.8614.1.S1_at	BQ838257	WRKY45 transcription factor n=1 Tax=Triticum aestivum RepID=A3RG93_WHEAT	2,237
Ta.4725.1.S1_at	CK195830	WRKY14 transcription factor n=1 Tax=Triticum aestivum RepID=B3GAU5_WHEAT	2,207
Ta.30495.1.A1_s_at	CK214893	CBFIVc-14.1 n=2 Tax=Triticum RepID=A0MPL5_WHEAT	2,176
Ta.21250.1.S1_at	BJ273029	MADS-box transcription factor TaAGL23 n=4 Tax=Triticeae RepID=Q1G185_WHEAT	2,144
TaAffx.80313.1.S1_at	CA698434	Putative WRKY5 protein (Fragment) n=1 Tax=Hordeum vulgare RepID=Q5W1F7_HORVU	2,094
Ta.22101.1.A1_at	CA721955	Chlorophyll a-b binding protein 22L, chloroplastic n=14 Tax=Solanaceae	-2,022
Ta.6374.3.S1_a_at	BJ300310	Squamosa promoter-binding-like protein 13 n=3 Tax=Oryza sativa RepID=SPL13_ORYSJ	-2,092
TaAffx.143995.17.A1_at	AY188331	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-2,714
Ta.9216.1.A1_a_at	BQ165936	Homeobox-leucine zipper protein HOX12 n=3 Tax=Oryza sativa RepID=HOX12_ORYSJ	-2,813
Ta.30640.1.S1_at	CD861747	Triticum aestivum flowering locus T mRNA, complete cds or VRN3	-3,250
Ta.246.1.S1_at	AF224499	Homeobox protein KNOX3 n=3 Tax=Triticeae RepID=KNOX3_HORVU	-3,477
Ta.9216.1.A1_x_at	BQ165936	Homeobox-leucine zipper protein HOX12 n=3 Tax=Oryza sativa RepID=HOX12_ORYSJ	-3,497
Ta.3583.1.A1_at	BJ219585	MADS-box protein 8 n=4 Tax=Pooideae RepID=Q9LEI0_HORVU	-5,963
TaAffx.120063.2.S1_s_at	BJ245749	MADS-box transcription factor 18 n=4 Tax=Oryza sativa RepID=MAD18_ORYSJ	-37,146
Ta.30607.1.A1_at	BJ264278	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-60,556
TaAffx.120063.1.S1_at	CF134093	MADS-box transcription factor 18 n=4 Tax=Oryza sativa RepID=MAD18_ORYSJ	-64,352
Ta.6793.1.A1_at	CD492136	MADS2 n=3 Tax=Triticeae RepID=A5X498_WHEAT	-105,103
TaAffx.143995.17.S1_s_at	AY188331	MADS box transcription factor n=15 Tax=Triticeae RepID=O82128_WHEAT	-108,744

Legend: According to Affymetrix Gene Chip® wheat genome array of the 26 Transcription factors Probe sets IDs differentially regulated (see Fig. 1) complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

Table S5.3 *mvp* wheat plant class 3 genes (sugar metabolism related genes) regulated identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
TaAffx.107485.1.S1_at	CA699183	Glucomannan 4-beta-mannosyltransferase 1 n=3 Tax=Oryza sativa RepID=CSLA1_ORYSJ	6,833
Ta.26048.1.S1_x_at	CD454944	Beta-glucanase n=1 Tax=Hordeum vulgare RepID=Q7M1K2_HORVU	6,647
TaAffx.15327.1.S1_at	AJ610775	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,959
Ta.22565.1.S1_at	BT009372	UDP-glucosyl transferase n=1 Tax=Triticum aestivum RepID=C5HUX8_WHEAT	3,453
TaAffx.24475.1.S1_at	CA718969	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,382
Ta.22565.1.S1_x_at	BT009372	UDP-glucosyl transferase n=1 Tax=Triticum aestivum RepID=C5HUX8_WHEAT	3,141
TaAffx.24475.1.S1_x_at	CA718969	Glucan endo-1,3-beta-glucosidase GII n=9 Tax=Triticeae RepID=E13B_HORVU	3,123
Ta.12517.1.S1_at	CK163074	Sugar transport protein 1 n=2 Tax=Zea mays RepID=Q6B4G9_MAIZE	2,900
Ta.3828.3.A1_x_at	BJ254343	Putative glucan endo-1,3-beta-D-glucosidase (Fragment) n=1 Tax=Triticum aestivum	2,596
Ta.223.1.S1_at	AF112965	Beta-1,3-glucanase n=1 Tax=Triticum aestivum RepID=Q9XEN5_WHEAT	2,464
TaAffx.20635.1.S1_at	CD895869	Sugar transporter family protein, expressed n=3 Tax=Oryza sativa RepID=Q10QG4_ORYSJ	2,319
Ta.3828.3.A1_a_at	BJ254343	Putative glucan endo-1,3-beta-D-glucosidase (Fragment) n=1 Tax=Triticum aestivum	2,018
TaAffx.15847.3.S1_at	BQ802374	Xyloglucan endotransglycosylase (XET) n=1 Tax=Hordeum vulgare RepID=P93671_HORVU	-2,200
Ta.9000.1.S1_at	CD453515	Acid beta-fructofuranosidase n=1 Tax=Triticum aestivum RepID=Q575T1_WHEAT	-2,378
Ta.9058.1.S1_at	CA642666	Acid beta-fructofuranosidase n=1 Tax=Triticum aestivum RepID=Q575T1_WHEAT	-3,184
TaAffx.410.1.S1_s_at	CA646394	Sucrose synthase metabolism (Fragment) n=3 Tax=Poaceae RepID=A6MZV1_ORYSI	-3,263
TaAffx.410.1.S1_at	CA646394	Sucrose synthase metabolism (Fragment) n=3 Tax=Poaceae RepID=A6MZV1_ORYSI	-3,519

Legend: According to Affymetrix Gene Chip® wheat genome array of the 17 sugar metabolism related Probe sets IDs differentially regulated (see Fig. 1) complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

Table S5. 4 *mvp* wheat plant class 4 genes (oxidative stress related genes) regulated identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
TaAffx.1074.1.S1_at	CK212638	Cytochrome c oxidase subunit 1 n=9 Tax=Aphidomorpha RepID=Q69HZ8_9HEMI	3,884
TaAffx.100029.1.S1_at	CA678048	Leucoanthocyanidin dioxygenase n=1 Tax=Zea mays RepID=B6U9L0_MAIZE	3,377
Ta.5235.1.S1_x_at	BG606752	Peroxidase n=2 Tax=Triticeae RepID=O49866_HORVU	3,056
Ta.5385.1.S1_at	X85228	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,933
TaAffx.50125.2.S1_at	CA745672	Cytochrome P450 n=1 Tax=Triticum aestivum RepID=Q9AVM3_WHEAT	2,707
TaAffx.85775.1.S1_at	CA621227	Cytochrome P450 monooxygenase CYP71U4v2 n=2 Tax=Hordeum vulgare RepID=Q52PG2_HORVD	2,680
Ta.24106.1.S1_x_at	CA665159	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,569
TaAffx.115935.1.S1_x_at	AY596267	Polyphenol oxidase n=2 Tax=Triticum aestivum RepID=C0SPI5_WHEAT	2,515
TaAffx.65294.1.A1_at	BJ252171	Chloroplast lipocalin n=2 Tax=Triticeae RepID=Q38JB3_WHEAT	2,437
Ta.3703.2.S1_s_at	BJ246711	Cytochrome P450 CYP709E4 n=2 Tax=Zea mays RepID=B6TF97_MAIZE	2,431
Ta.20570.1.A1_at	CA741536	1-aminocyclopropane-1-carboxylate oxidase (Fragment) n=2 Tax=Andropogoneae RepID=O81607_SORBI	2,411
Ta.23366.3.A1_x_at	BQ161967	Class III peroxidase 62 n=2 Tax=Oryza sativa Japonica Group RepID=Q5U1N1_ORYSJ	2,400
TaAffx.80306.1.S1_at	CA698528	1-aminocyclopropane-1-carboxylate oxidase (Fragment) n=1 Tax=Triticum monococcum	2,322
Ta.23366.3.A1_at	BQ161967	Class III peroxidase 62 n=2 Tax=Oryza sativa Japonica Group RepID=Q5U1N1_ORYSJ	2,290
Ta.29496.1.S1_x_at	CK195383	Peroxidase 12 n=2 Tax=Zea mays RepID=B6THG0_MAIZE	2,281
Ta.21505.1.S1_at	CK213957	Root peroxidase n=1 Tax=Triticum aestivum RepID=B4F6E8_WHEAT	2,276
Ta.21307.1.S1_x_at	CK199589	Peroxidase 12 n=2 Tax=Zea mays RepID=B6THG0_MAIZE	2,186
Ta.303.3.S1_x_at	AJ610447	Glutathione S-transferase 2 n=2 Tax=Triticum aestivum RepID=GSTF2_WHEAT	2,175
Ta.24710.1.S1_at	CA678411	Peroxidase 1 n=8 Tax=Triticeae RepID=PER1_HORVU	2,087
Ta.10549.2.A1_at	BQ165963	Alternative oxidase n=3 Tax=Oryza sativa RepID=O82807_ORYSJ	2,050
Ta.19609.1.S1_at	AJ615628	Cytochrome P450 n=1 Tax=Triticum aestivum RepID=Q2V065_WHEAT	2,015

Legend: According to Affymetrix Gene Chip® wheat genome array of the 21 oxidative stress related Probe sets IDs up-regulated complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented in decreasing order of differential expression of ≥ 2 fold cut off and p-value $p \leq 0,05$.

Table S5. 5 *mvp* wheat plant class 5 of miscellaneous regulated genes identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
TaAffx.57571.1.S1_at	CA626944	Apyrase n=1 Tax=Lolium perenne RepID=B9U140_LOLPR	31,363
TaAffx.70677.1.S1_at	CA701525	Chaperone protein dnaJ n=2 Tax=Andropogoneae RepID=B4FBY1_MAIZE	7,753
TaAffx.89610.1.S1_at	AL823263	Agmatine coumaroyltransferase n=1 Tax=Zea mays RepID=B6ST92_MAIZE	6,540
Ta.27314.1.S1_at	BT009398	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays	6,180
Ta.8902.2.S1_at	CA640147	Taxane 13-alpha-hydroxylase n=1 Tax=Zea mays RepID=B6SYR0_MAIZE	5,532
Ta.28917.2.S1_a_at	CA637102	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	4,341
Ta.25531.1.A1_at	CD373817	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	4,302
Ta.18720.1.S1_x_at	BJ288588	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	3,753
Ta.8902.1.S1_at	BQ162604	Taxane 13-alpha-hydroxylase n=1 Tax=Zea mays RepID=B6SYR0_MAIZE	3,703
TaAffx.132143.1.S1_s_at	CK212211	Cyanate hydratase n=3 Tax=Oryza sativa RepID=CYNS_ORYSJ	3,432
Ta.25531.2.A1_x_at	CA680295	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	3,426
Ta.9220.1.S1_a_at	CK210556	Putative phenylalanine ammonia-lyase (Fragment) n=1 Tax=Hordeum vulgare	3,352
TaAffx.70601.1.S1_at	BQ802878	Triticum aestivum cultivar 92R137 PDR-type ABC transporter (PDR1)	3,344
Ta.8232.1.A1_at	BQ161629	PDR-type ABC transporter n=2 Tax=Triticum aestivum RepID=B9UYP3_WHEAT	3,309
TaAffx.97211.1.S1_at	AJ614654	O-methyltransferase n=1 Tax=Triticum aestivum RepID=B4ERX7_WHEAT	3,268
Ta.13784.1.S1_at	AL823038	BLT14.1 protein n=2 Tax=Triticeae RepID=Q40032_HORVU	3,178
TaAffx.110222.1.S1_x_at	CA661932	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays RepID=B6STG1_MAIZE	3,144
Ta.28917.1.S1_at	CK166116	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	3,099
Ta.21646.1.S1_x_at	CA686121	Non-specific lipid-transfer protein n=3 Tax=Oryza sativa RepID=Q75GN2_ORYSJ	3,071
Ta.28917.1.S1_x_at	CK166116	Cold acclimation protein WCOR518 (Fragment) n=1 Tax=Triticum aestivum RepID=P93611_WHEAT	2,978
TaAffx.113315.1.S1_at	CA615901	Iron-phytosiderophore transporter n=1 Tax=Hordeum vulgare RepID=Q2PGC4_HORVU	2,967
Ta.25531.2.A1_at	CA680295	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	2,964
Ta.30921.1.S1_x_at	CN012317	12-oxo-phytodienoic acid reductase n=1 Tax=Zea mays RepID=Q49HE1_MAIZE	2,948
Ta.24730.2.S1_x_at	BE430562	Protochlorophyllide reductase (Fragment) n=4 Tax=Poaceae RepID=POR_AVESA	2,743
TaAffx.16032.1.A1_at	BQ800652	Cell wall invertase n=1 Tax=Lolium perenne RepID=Q2QI10_LOLPR	2,730
Ta.28613.1.S1_at	U73213	Cold acclimation protein WCOR726 n=3 Tax=Triticeae RepID=P93610_WHEAT	2,708
TaAffx.120890.1.S1_x_at	CA688266	Serine/threonine kinase-like protein n=3 Tax=Andropogoneae RepID=B6SUE5_MAIZE	2,575
Ta.11016.1.S1_at	BQ168385	Calmodulin-like protein n=1 Tax=Zea mays RepID=B6TXW9_MAIZE	2,565
TaAffx.55592.1.S1_at	CA663917	Potassium transporter 5 n=1 Tax=Oryza sativa Japonica Group RepID=HAK5_ORYSJ	2,481
Ta.21272.1.S1_at	CA668970	Cell division AAA ATPase family protein n=10 Tax=Triticeae RepID=B6Z264_WHEAT	2,470
Ta.8228.1.S1_at	BQ161624	Agmatine coumaroyltransferase n=3 Tax=Hordeum vulgare RepID=A9ZPJ6_HORVU	2,432
Ta.351.2.S1_x_at	CA673444	Cold acclimation induced protein 2-1 n=1 Tax=Triticum aestivum RepID=Q4KXE0_WHEAT	2,400
Ta.23397.1.S1_x_at	BJ292777	Glycine-rich cell wall structural protein n=1 Tax=Hordeum vulgare RepID=GRP1_HORVU	2,390
Ta.23397.2.S1_x_at	CA718270	Pherophorin-dz1 protein n=1 Tax=Volvox carteri f. nagariensis RepID=Q8L685_VOLCA	2,384

Ta.28659.1.S1_x_at	CA670381	Putative protease inhibitor n=1 Tax=Hordeum vulgare RepID=Q96465_HORVU	2,384
Ta.18720.3.S1_x_at	BJ295779	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	2,357
Ta.21650.1.A1_at	CA667760	NBS-LRR resistance-like protein n=1 Tax=Hordeum vulgare RepID=A9UKM2_HORVU	2,343
Ta.24254.1.S1_a_at	CA616263	Plastid omega-3 fatty acid desaturase n=2 Tax=Oryza sativa Japonica Group RepID=Q2HWS9_ORYSJ	2,319
Ta.18720.1.S1_a_at	BJ288588	Gamma-thionin n=1 Tax=Hordeum vulgare RepID=Q39999_HORVU	2,267
Ta.8512.1.S1_at	BQ162001	Os07g0631700 protein n=2 Tax=Oryza sativa RepID=Q8LHN5_ORYSJ	2,254
Ta.6187.1.S1_at	BQ167028	Glycine-rich cell wall structural protein n=2 Tax=Zea mays RepID=B6ST85_MAIZE	2,249
Ta.351.1.S1_at	CA669389	Cold acclimation induced protein 2-1 n=1 Tax=Triticum aestivum RepID=Q4KXE0_WHEAT	2,247
TaAffx.113701.1.S1_s_at	CA606782	Pleiotropic drug resistance protein 4 n=3 Tax=Oryza sativa RepID=PDR4_ORYSJ	2,172
Ta.169.1.S1_x_at	CA652856	Germin-like 12 n=7 Tax=Triticeae RepID=Q43487_HORVU	2,170
TaAffx.3462.1.S1_at	CK215505	Early salt stress and cold acclimation-induced protein 2-1 n=1 Tax=Lophopyrum elongatum	2,161
Ta.27506.1.S1_at	AY428038	Ammonium transporter AMT2.1 n=1 Tax=Triticum aestivum RepID=Q6T8L6_WHEAT	2,155
Ta.2793.1.S1_at	AB055077	Multidrug resistance protein 1 homolog n=1 Tax=Triticum aestivum RepID=Q8RVT7_WHEAT	2,151
TaAffx.77715.1.S1_at	CA744342	S-domain receptor-like protein kinase n=1 Tax=Oryza granulata RepID=B9V0L1_9ORYZ	2,151
Ta.6990.1.S1_at	CA611113	Pleiotropic drug resistance protein 4 n=3 Tax=Oryza sativa RepID=PDR4_ORYSJ	2,133
TaAffx.81369.1.S1_at	CA686927	Receptor-like protein kinase n=2 Tax=Oryza sativa RepID=Q9M575_ORYSA	2,099
Ta.27258.1.S1_at	BT009386	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 n=1 Tax=Zea mays	2,091
Ta.14837.1.S1_at	CA682925	Calreticulin, putative n=1 Tax=Ricinus communis RepID=B9RM48_RICCO	2,075
Ta.3154.1.S1_at	BJ223797	Sorghum bicolor hypothetical protein (SORBIDRAFT_0019s004610) mRNA	2,043
Ta.24723.1.S1_x_at	CA667447	PSBGer1 protein n=1 Tax=Triticum aestivum RepID=P93598_WHEAT	2,043
TaAffx.56501.1.S1_at	BU099852	Sorghum bicolor hypothetical protein (SORBIDRAFT_0019s004610) mRNA, complete cds	2,026
TaAffx.128683.1.S1_x_at	CA684810	Cell division AAA ATPase family protein n=10 Tax=Triticeae RepID=B6Z264_WHEAT	2,022
Ta.26236.1.A1_at	CD453043	Abhydrolase domain-containing protein 5 n=2 Tax=Andropogoneae RepID=B4FAQ7_MAIZE	2,005
Ta.28827.1.S1_at	BQ800700	Histone H1 n=2 Tax=Triticum aestivum RepID=O65795_WHEAT	2,004
Ta.1282.4.S1_at	CA635994	Non-specific lipid-transfer protein n=3 Tax=Triticeae RepID=Q42848_HORVU	-2,017
TaAffx.95414.1.S1_at	BQ803340	Branched-chain-amino-acid aminotransferase n=1 Tax=Hordeum vulgare	-2,020
Ta.2969.1.A1_at	CK165153	Expansin EXPB2 n=1 Tax=Triticum aestivum RepID=Q6QFA2_WHEAT	-2,024
Ta.28728.1.S1_at	AF139815	Aquaporin PIP2-4 n=9 Tax=Poaceae RepID=PIP24_MAIZE	-2,035
TaAffx.113526.2.S1_s_at	CA723504	Proline-rich protein n=2 Tax=Triticum aestivum RepID=C7EZF0_WHEAT	-2,081
Ta.28728.1.S1_x_at	AF139815	Aquaporin PIP2-4 n=9 Tax=Poaceae RepID=PIP24_MAIZE	-2,093
TaAffx.8804.2.S1_at	BE217049	Probable aquaporin PIP2-7 n=3 Tax=Oryza sativa RepID=PIP27_ORYSJ	-2,144
Ta.30144.1.A1_x_at	BQ166180	Proline-rich protein n=1 Tax=Sorghum bicolor RepID=A7KH48_SORBI	-2,190
Ta.3.1.S1_at	BJ265463	Beta-amylase n=2 Tax=Triticeae RepID=AMYB_WHEAT	-2,226
TaAffx.43693.1.S1_at	BF199968	Xet3 protein n=1 Tax=Festuca pratensis RepID=Q949H9_FESPR	-2,268
TaAffx.71208.1.S1_at	BE492167	Amidase n=2 Tax=Zea mays RepID=B6TMI1_MAIZE	-2,269
TaAffx.8804.1.S1_s_at	CK164001	Probable aquaporin PIP2-7 n=3 Tax=Oryza sativa RepID=PIP27_ORYSJ	-2,287
Ta.13160.1.S1_at	CK195065	Stem-specific protein n=4 Tax=Andropogoneae RepID=Q5U7K5_9POAL	-2,289

Ta.1600.1.A1_at	CK164799	Nodulin-like protein 5NG4 n=3 Tax=Andropogoneae RepID=B6TLA9_MAIZE	-2,301
Ta.10400.1.S1_at	BJ251360	Cortical cell-delineating protein n=7 Tax=Zea mays RepID=B6SIN6_MAIZE	-2,324
TaAffx.632.1.A1_at	CD490405	Hordeum vulgare ENOD40-like protein mRNA, complete cds	-2,325
TaAffx.36760.1.S1_at	BJ315664	CEN-like protein 2, putative, expressed n=13 Tax=Poaceae RepID=Q53Q71_ORYSJ	-2,392
Ta.346.1.A1_at	BJ254518	Cycloartenol synthase, putative, expressed n=1 Tax=Oryza sativa Japonica Group	-2,616
TaAffx.16936.1.S1_at	CK216481	Fasciclin-like protein FLA16 n=2 Tax=Triticum aestivum RepID=Q06I90_WHEAT	-2,665
Ta.20696.3.S1_x_at	CA598744	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-2,767
Ta.438.2.S1_x_at	CK212622	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,276
Ta.22954.1.S1_at	CA673824	Putative thionin Osthi1 n=2 Tax=Oryza sativa RepID=Q5Z4W6_ORYSJ	-3,384
Ta.438.1.S1_x_at	BG908651	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,451
Ta.22954.2.S1_x_at	CA675346	gene="ACT-1" actin [Triticum aestivum] protein_id="AAW78915.1	-3,497
Ta.438.1.S1_a_at	BG908651	Nuclease PA3 n=3 Tax=Zea mays RepID=B6U2F0_MAIZE	-3,696
Ta.22954.1.S1_a_at	CA673824	Putative thionin Osthi1 n=2 Tax=Oryza sativa RepID=Q5Z4W6_ORYSJ	-3,839
Ta.28186.2.A1_a_at	CA671332	Voltage-dependent outwardly rectifying plasma membrane K+ channel KCO1/TPK1 n=4	-5,131
Ta.20696.3.S1_s_at	CA598744	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-6,406
TaAffx.36593.1.S1_at	BQ166396	Amylase inhibitor-like protein n=3 Tax=Triticum RepID=A9UID9_WHEAT	-8,802
TaAffx.116496.1.S1_at	CA693971	Phytochrome C n=10 Tax=Triticeae RepID=Q2I714_HORVD	-14,526
Ta.21379.1.S1_s_at	CA674527	Voltage-dependent outwardly rectifying plasma membrane K+ channel KCO1/TPK1 n=4	-15,607
Ta.28005.1.A1_at	CD862101	Phytochrome C (Fragment) n=1 Tax=Hordeum vulgare RepID=Q945T7_HORVU	-16,267
Ta.2948I.1.S1_at	CK194207	Circumsporozoite protein n=2 Tax=Andropogoneae RepID=B6TF33_MAIZE	-169,064

Legend: According to Affymetrix Gene Chip® wheat genome array of the 91 miscellaneous regulated genes, Probe setst IDs up-regulated complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

Table S5.6 *mvp* wheat plant class 6 regulated genes (unknown genes) identified by microarray profiling

Affymetrix Probe sets IDs	GenBank Accession	UniProt and NCBI Description	Fold Change
Ta.8447.1.S1_a_at	CA669038	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8AKC8_ORYSI	12,977
Ta.19723.1.S1_at	CA657361	wlm0.pk0034.h7 wlm0 Triticum aestivum cDNA clone wlm0.pk0034.h7 5' end	6,056
TaAffx.109794.1.S1_s_at	CA668708	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8AKC8_ORYSI	5,763
Ta.62.1.S1_x_at	BM136002	Os01g0382000 protein n=5 Tax=Oryza sativa RepID=Q7F2P0_ORYSJ	5,324
TaAffx.110724.1.S1_at	CA655297	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B8A3M2_MAIZE	5,219
Ta.28233.1.S1_at	CA599187	Putative uncharacterized protein Sb10g024350 n=1 Tax=Sorghum bicolor RepID=C5Z653_SORBI	5,214
Ta.23271.1.S1_s_at	CA680274	wlm24.pk0004.a5 wlm24 Triticum aestivum cDNA clone wlm24.pk0004.a5 5' end	5,156
Ta.8356.1.S1_at	BQ161783	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKT6_ORYSI	5,002
Ta.30765.1.S1_at	CN011347	Putative uncharacterized protein Sb01g037040 n=1 Tax=Sorghum bicolor RepID=C5X1U8_SORBI	4,935
TaAffx.53867.1.S1_at	CA688277	wlm96.pk039.j13 wlm96 Triticum aestivum cDNA clone wlm96.pk039.j13 5' end	4,499
Ta.23271.2.S1_a_at	CA677139	wlm12.pk0009.c12 wlm12 Triticum aestivum cDNA clone wlm12.pk0009.c12 5' end	4,484
TaAffx.61466.1.S1_at	BJ251396	Putative uncharacterized protein Sb03g037575 n=1 Tax=Sorghum bicolor RepID=C5XN35_SORBI	4,472
TaAffx.97767.1.A1_at	CA721990	Putative uncharacterized protein Sb01g033530 n=1 Tax=Sorghum bicolor RepID=C5WXP8_SORBI	4,222
Ta.21340.1.S1_a_at	CA683606	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=Q6ZKP5_ORYSJ	4,203
Ta.8356.1.S1_s_at	BQ161783	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKT6_ORYSI	4,191
TaAffx.7302.1.S1_at	CA662601	Os01g0678000 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q5QM71_ORYSJ	4,055
TaAffx.27177.1.S1_at	CA680302	Putative uncharacterized protein Sb09g004960 n=1 Tax=Sorghum bicolor RepID=C5Z115_SORBI	3,985
Ta.23165.2.S1_x_at	CA667728	Triticum aestivum clone wlsu2.pk0001.h3:fis, full insert mRNA sequence	3,958
TaAffx.111759.3.S1_s_at	CA660613	wlm1.pk0023.g12 wlm1 Triticum aestivum cDNA clone wlm1.pk0023.g12 5' end	3,918
Ta.23165.3.S1_x_at	CA669496	Triticum aestivum clone wlsu2.pk0001.h3:fis, full insert mRNA sequence	3,912
TaAffx.43393.1.S1_at	BQ482808	Os02g0102900 protein n=4 Tax=Poaceae RepID=Q6ZFJ9_ORYSJ	3,861
Ta.192.1.S1_at	U32431	Putative uncharacterized protein n=1 Tax=Triticum aestivum RepID=Q41523_WHEAT	3,853
Ta.14779.1.S1_at	CA681945	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	3,794
Ta.27503.1.A1_at	CA659276	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8B370_ORYSI	3,794
Ta.12198.1.A1_at	BQ171803	WHE1659-1662_P05_P05ZT Wheat heat stressed flag leaf cDNA library Triticum aestivum	3,723
Ta.9430.1.S1_at	AJ614438	Putative uncharacterized protein Sb10g004170 n=1 Tax=Sorghum bicolor RepID=C5Z4H2_SORBI	3,685
Ta.19805.2.S1_a_at	CA683372	Os04g0168400 protein n=3 Tax=Oryza sativa RepID=B7F8V8_ORYSJ	3,659
Ta.12118.1.S1_a_at	CA698971	Putative uncharacterized protein n=1 Tax=Zea mays RepID=C0PFC3_MAIZE	3,606
TaAffx.97737.1.A1_at	CA722456	Triticum aestivum cDNA, clone: WT006_G18, cultivar: Chinese Spring	3,559
TaAffx.122333.1.S1_at	CA725295	Putative uncharacterized protein Sb03g045090 n=1 Tax=Sorghum bicolor RepID=C5XH43_SORBI	3,539
TaAffx.26668.1.S1_at	CA686407	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	3,528
TaAffx.13303.1.S1_at	BG909514	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2WKZ0_ORYSI	3,437
Ta.520.1.S1_at	BE604553	WHE1413-1416_P07_P07ZS Wheat drought stressed leaf cDNA library Triticum aestivum	3,429

TaAffx.108556.1.S1_at	CA692789	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B4FS23_MAIZE	3,396
Ta.5766.1.S1_at	BJ289079	Os03g0218400 protein n=5 Tax=Poaceae RepID=Q10PW9_ORYSJ	3,376
Ta.7711.1.A1_at	BQ161212	Putative uncharacterized protein Sb08g017560 n=1 Tax=Sorghum bicolor RepID=C5YPJ9_SORBI	3,359
Ta.231.1.S1_x_at	AF079526	PR17c n=2 Tax=Triticeae RepID=A7YA60_HORVD	3,356
TaAffx.26815.1.S1_at	CA684496	BLN1-2 n=3 Tax=Hordeum vulgare subsp. vulgare RepID=B8X453_HORVD	3,336
TaAffx.128798.2.S1_x_at	CA695322	Putative uncharacterized protein Sb08g017540 n=1 Tax=Sorghum bicolor RepID=C5YPJ7_SORBI	3,319
Ta.3869.1.S1_at	CA674403	Os08g0127100 protein n=6 Tax=Poaceae RepID=Q6ZK52_ORYSJ	3,266
TaAffx.55188.1.S1_at	CA670456	wlsu1.pk026.n9 wlsu1 Triticum aestivum cDNA clone wlsu1.pk026.n9 5' end	3,249
Ta.6051.1.S1_a_at	CD914245	Os10g0416500 protein n=3 Tax=Oryza sativa RepID=Q9FYR9_ORYSJ	3,239
Ta.20549.1.S1_x_at	CA668159	Putative uncharacterized protein Sb04g030310 n=1 Tax=Sorghum bicolor RepID=C5Y093_SORBI	3,227
TaAffx.81496.1.S1_at	CA685342	Putative uncharacterized protein Sb05g019490 n=1 Tax=Sorghum bicolor RepID=C5Y384_SORBI	3,220
Ta.30860.1.S1_at	CN010964	WHE3878_C08_F16ZS Wheat Fusarium graminearum infected spike cDNA Triticum aestivum	3,203
Ta.27279.1.S1_at	BT009316	Os06g0330400 protein n=2 Tax=Oryza sativa RepID=Q69UX2_ORYSJ	3,114
TaAffx.108556.1.S1_x_at	CA692789	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B4FS23_MAIZE	3,113
TaAffx.84552.1.S1_at	CA634441	wle1n.pk0086.g12 wle1n Triticum aestivum cDNA clone wle1n.pk0086.g12 5' end	3,096
TaAffx.71225.1.A1_at	BE492103	WHE0551_F06_F06ZE Triticum monococcum vegetative apex cDNA Triticum monococcum	3,073
Ta.21326.1.S1_a_at	AJ613350	Os07g0518100 protein n=3 Tax=Oryza sativa RepID=Q7EZ52_ORYSJ	3,052
Ta.10617.1.S1_at	BQ166125	Os01g0108400 protein n=4 Tax=Oryza sativa RepID=Q0JRC6_ORYSJ	3,002
Ta.6051.3.S1_x_at	CA717933	Os10g0416500 protein n=3 Tax=Oryza sativa RepID=Q9FYR9_ORYSJ	2,956
Ta.28233.2.S1_a_at	CA695499	Putative uncharacterized protein Sb10g024350 n=1 Tax=Sorghum bicolor RepID=C5Z653_SORBI	2,934
TaAffx.114390.1.S1_at	CA593923	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B6SJC9_MAIZE	2,750
Ta.13991.1.S1_x_at	CA666889	Putative uncharacterized protein Sb03g043610 n=1 Tax=Sorghum bicolor RepID=C5XG61_SORBI	2,750
Ta.9332.1.S1_x_at	BQ789066	Os09g0441400 protein n=2 Tax=Oryza sativa RepID=Q69P73_ORYSJ	2,643
Ta.3869.2.S1_at	CA735969	Triticum aestivum cDNA, clone: WT008_I09, cultivar: Chinese Spring	2,636
Ta.20549.1.S1_s_at	CA668159	Putative uncharacterized protein Sb04g030310 n=1 Tax=Sorghum bicolor RepID=C5Y093_SORBI	2,621
Ta.12434.1.S1_at	CD862952	OSJNBa0086B14.7 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q7XV48_ORYSJ	2,618
TaAffx.81921.1.S1_at	CA680100	Os01g0613500 protein n=2 Tax=Oryza sativa RepID=Q9FTI3_ORYSJ	2,617
TaAffx.15958.1.S1_at	BQ801634	Os01g0914100 protein n=2 Tax=Oryza sativa RepID=Q8S077_ORYSJ	2,595
Ta.13956.1.S1_at	BJ287532	cDNA library, Wh_r Triticum aestivum cDNA clone whr20i23 3'	2,559
Ta.10581.1.A1_at	CK156211	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf14j06, mRNA sequence	2,552
TaAffx.24109.1.A1_at	CK216113	Os03g0400200 protein n=4 Tax=Oryza sativa RepID=Q10K20_ORYSJ	2,547
Ta.231.1.S1_at	AF079526	PR17c n=2 Tax=Triticeae RepID=A7YA60_HORVD	2,547
TaAffx.124475.1.A1_at	CK212125	BLT14.2 protein n=2 Tax=Hordeum vulgare RepID=Q40033_HORVU	2,528
Ta.11397.1.A1_at	BQ170011	Putative uncharacterized protein Sb02g035400 n=1 Tax=Sorghum bicolor RepID=C5XAR6_SORBI	2,498
Ta.30913.1.A1_at	CN012655	Os09g0454600 protein n=3 Tax=Oryza sativa RepID=Q0J198_ORYSJ	2,461
Ta.22628.1.S1_x_at	CA690208	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B6SP49_MAIZE	2,453
Ta.22628.1.S1_at	CA690208	Putative uncharacterized protein n=1 Tax=Zea mays RepID=B6SP49_MAIZE	2,436

TaAffx.109085.1.S1_at	CA680382	wlm24.pk0006.g11 wlm24 Triticum aestivum cDNA clone wlm24.pk0006.g11 5' end	2,417
TaAffx.99316.1.A1_at	CK207671	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9GE50_ORYSJ	2,412
Ta.20197.1.S1_at	CA674805	Putative uncharacterized protein (Fragment) n=2 Tax=Papilionoideae RepID=B7FFL2_MEDTR	2,410
TaAffx.34169.1.S1_at	BJ287371	Putative uncharacterized protein n=2 Tax=Oryza sativa RepID=B9FBJ1_ORYSJ	2,410
Ta.11421.1.A1_at	BQ170075	Os01g0266500 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q9SDD7_ORYSJ	2,401
Ta.25487.1.S1_at	CD373987	Putative uncharacterized protein Sb02g035450 n=1 Tax=Sorghum bicolor RepID=C5XAS1_SORBI	2,385
Ta.25539.1.S1_at	CA678031	BLT14.1 protein n=2 Tax=Triticeae RepID=Q40032_HORVU	2,362
Ta.4696.1.S1_at	BJ275186	Os01g0366300 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q5Z8B7_ORYSJ	2,353
TaAffx.51261.1.S1_at	CA728497	Os01g0793900 protein n=2 Tax=Oryza sativa RepID=Q8S1K8_ORYSJ	2,329
Ta.19786.1.A1_at	CA658969	Putative uncharacterized protein Sb01g016640 n=1 Tax=Sorghum bicolor RepID=C5WV89_SORBI	2,294
Ta.25754.1.A1_at	CD373766	Putative uncharacterized protein Sb03g045850 n=1 Tax=Sorghum bicolor RepID=C5XHV9_SORBI	2,291
Ta.3162.1.S1_at	BJ215513	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FSW5_ORYSJ	2,286
Ta.25542.1.S1_at	CD896341	Os02g0712700 protein n=3 Tax=Oryza sativa RepID=Q0DY65_ORYSJ	2,273
Ta.8399.2.S1_at	CA694080	Putative uncharacterized protein Sb03g028700 n=1 Tax=Sorghum bicolor RepID=C5XEF4_SORBI	2,266
Ta.8512.1.S1_at	BQ162001	Os07g0631700 protein n=2 Tax=Oryza sativa RepID=Q8LHN5_ORYSJ	2,254
TaAffx.82108.1.S1_x_at	CA677464	Os01g0115700 n=1 Tax=Oryza sativa Japonica Group RepID=UPI0000DD891C	2,249
Ta.24254.2.S1_at	BQ800827	Os03g0290300 protein (Fragment) n=1 Tax=Oryza sativa Japonica Group RepID=Q0DSS9_ORYSJ	2,246
Ta.13232.2.S1_at	BQ166297	WHE0840_F03_L06ZT Wheat vernalized crown cDNA library Triticum aestivum	2,240
TaAffx.131248.2.S1_at	CA670789	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf2f01, mRNA sequence	2,223
TaAffx.83027.1.S1_at	CA662924	Os05g0135100 protein n=1 Tax=Oryza sativa Japonica Group RepID=Q0DKY8_ORYSJ	2,221
Ta.4921.1.S1_at	BE438217	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FAG9_ORYSJ	2,216
Ta.15129.1.S1_at	CA690804	wlm96.pk052.p8 wlm96 Triticum aestivum cDNA clone wlm96.pk052.p8 5' end	2,215
TaAffx.28302.4.S1_at	CA662104	wlmk1.pk0015.d5 wlmk1 Triticum aestivum cDNA clone wlmk1.pk0015.d5 5' end	2,208
Ta.24832.1.S1_s_at	CA668285	Os08g0127100 protein n=6 Tax=Poaceae RepID=Q6ZK52_ORYSJ	2,182
TaAffx.16900.1.A1_at	CK215179	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=Q7X669_ORYSJ	2,180
Ta.22625.1.A1_s_at	CA666706	Os02g0634700 protein n=2 Tax=Oryza sativa RepID=Q6H7I7_ORYSJ	2,179
Ta.21035.1.S1_at	CA614540	Putative uncharacterized protein Sb03g006590 n=1 Tax=Sorghum bicolor RepID=C5XQN8_SORBI	2,166
TaAffx.131248.2.S1_s_at	CA670789	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf2f01, mRNA sequence	2,165
TaAffx.21249.1.S1_at	CA706624	Os02g0720600 protein n=2 Tax=Oryza sativa RepID=Q6Z674_ORYSJ	2,162
Ta.12671.1.S1_a_at	CK194385	Putative uncharacterized protein Sb04g024020 n=1 Tax=Sorghum bicolor RepID=C5XVR6_SORBI	2,160
TaAffx.5899.1.S1_at	CA696250	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=A2YM74_ORYSI	2,156
TaAffx.108908.1.S1_x_at	CA684616	Os03g0663500 protein (Fragment) n=3 Tax=Oryza sativa RepID=Q0DPU1_ORYSJ	2,153
Ta.27455.1.S1_at	CA644954	Putative uncharacterized protein Sb01g027360 n=1 Tax=Sorghum bicolor RepID=C5WPZ1_SORBI	2,152
Ta.12441.1.A1_at	BQ172342	Putative uncharacterized protein n=3 Tax=Zea mays RepID=C4JAI5_MAIZE	2,137
Ta.8076.1.S1_at	CA684491	Os02g0634700 protein n=2 Tax=Oryza sativa RepID=Q6H7I7_ORYSJ	2,135
Ta.28224.1.S1_x_at	CK193135	Os01g0384800 protein n=2 Tax=Oryza sativa RepID=Q5VNY3_ORYSJ	2,134

TaAffx.21249.1.S1_x_at	CA706624	Os02g0720600 protein n=2 Tax=Oryza sativa RepID=Q6Z674_ORYSJ	2,133
TaAffx.82108.1.S1_at	CA677464	Os01g0115700 n=1 Tax=Oryza sativa Japonica Group RepID=UPI0000DD891C	2,131
Ta.5824.2.S1_x_at	AL830800	Os02g0740600 protein n=2 Tax=Oryza sativa RepID=Q6Z7S9_ORYSJ	2,124
TaAffx.54530.1.S1_at	CA633759	Os04g0103500 protein n=2 Tax=Oryza sativa RepID=Q7XMR2_ORYSJ	2,122
Ta.8323.1.A1_at	BQ161741	Os02g0515200 protein n=2 Tax=Oryza sativa RepID=Q67UP1_ORYSJ	2,090
TaAffx.83591.1.S1_at	CA655732	Putative uncharacterized protein n=1 Tax=Oryza sativa Indica Group RepID=B8B433_ORYSI	2,087
TaAffx.8335.1.S1_at	CA627027	wl1n.pk151.c8 wl1n Triticum aestivum cDNA clone wl1n.pk151.c8 5' end	2,068
Ta.25845.1.S1_at	CD453621	Putative uncharacterized protein Sb09g003460 n=1 Tax=Sorghum bicolor RepID=C5Z001_SORBI	2,052
TaAffx.15674.1.A1_at	CD490328	WHE2494_H02_P04ZT Triticum monococcum DV92 early reproductive apex	2,044
Ta.4831.1.S1_at	AJ611741	Putative uncharacterized protein Sb06g032110 n=1 Tax=Sorghum bicolor RepID=C5YA11_SORBI	2,042
Ta.1830.2.S1_x_at	CA683961	Putative uncharacterized protein Sb01g030980 n=1 Tax=Sorghum bicolor RepID=C5WUJ5_SORBI	2,037
Ta.21711.1.S1_at	CD883645	Putative uncharacterized protein Sb07g020050 n=1 Tax=Sorghum bicolor RepID=C5YKT1_SORBI	2,028
TaAffx.31754.1.S1_at	CA608501	Putative uncharacterized protein n=1 Tax=Oryza sativa Japonica Group RepID=B9FXP2_ORYSJ	2,020
TaAffx.70641.1.S1_at	BQ802487	WHE2826_E02_I04ZS Triticum monococcum vernalized apex	2,018
TaAffx.4290.1.A1_at	CA681239	wlm24.pk0014.c7 wlm24 Triticum aestivum cDNA clone wlm24.pk0014.c7 5' end.	2,001
TaAffx.130076.1.S1_at	BQ168759	Os05g0550600 protein n=2 Tax=Oryza sativa RepID=Q6L4G9_ORYSJ	-2,002
TaAffx.18447.3.S1_s_at	CA737476	Putative uncharacterized protein Sb08g020600 n=1 Tax=Sorghum bicolor	-2,022
Ta.2926.1.A1_at	BJ207389	Triticum aestivum cDNA, clone: WT009_B01, cultivar: Chinese Spring	-2,022
Ta.28728.1.S1_at	AF139815	Aquaporin PIP2-4 n=9 Tax=Poaceae RepID=PIP24_MAIZE	-2,035
Ta.8640.1.S1_a_at	BQ807183	Os07g0169600 protein n=2 Tax=Oryza sativa RepID=Q69LD9_ORYSJ	-2,037
Ta.6558.1.S1_x_at	CK208447	Os03g0787200 protein n=2 Tax=Oryza sativa RepID=Q6F3B1_ORYSJ	-2,042
Ta.28847.1.S1_a_at	CA740446	Os01g0795100 protein n=2 Tax=Oryza sativa Japonica Group RepID=Q8S110_ORYSJ	-2,056
Ta.20938.2.A1_x_at	CA624118	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,066
TaAffx.63920.1.A1_x_at	BQ162587	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf149n21, mRNA sequence	-2,084
Ta.3857.1.A1_at	CA698230	Os08g0137400 protein n=3 Tax=Oryza sativa RepID=Q0J845_ORYSJ	-2,090
TaAffx.8097.1.S1_x_at	CA636056	wle1n.pk0106.b2 wle1n Triticum aestivum cDNA clone wle1n.pk0106.b2 5' end	-2,125
Ta.28422.1.A1_s_at	BJ317142	Os03g0724600 protein (Fragment) n=3 Tax=Oryza sativa RepID=Q0DP01_ORYSJ	-2,141
Ta.7724.3.S1_at	CA631315	Putative uncharacterized protein Sb09g001060 n=1 Tax=Sorghum bicolor	-2,145
Ta.8710.1.A1_at	BQ162259	Triticum aestivum cDNA, clone: WT012_L15, cultivar: Chinese Spring	-2,167
Ta.6934.1.A1_x_at	CK209084	UPI0000D8C4F1 related cluster n=1 Tax=Danio rerio RepID=UPI0000D8C4F1	-2,170
Ta.20540.3.S1_x_at	BJ318550	Putative uncharacterized protein Sb01g004270 n=2 Tax=Andropogoneae	-2,210
Ta.20540.3.S1_at	BJ318550	Putative uncharacterized protein Sb01g004270 n=2 Tax=Andropogoneae	-2,229
Ta.11684.1.A1_at	BQ170589	Os03g0234900 protein n=2 Tax=Oryza sativa RepID=Q5U1Q4_ORYSJ	-2,240
Ta.20938.1.A1_at	BQ169082	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,241
Ta.20938.1.A1_a_at	BQ169082	Os01g0117900 protein n=2 Tax=Oryza sativa RepID=Q9FTZ6_ORYSJ	-2,271
TaAffx.16068.1.S1_at	BQ168935	Oryza sativa Japonica Group cDNA clone:002-112-B01, full insert sequence	-2,273
Ta.7724.3.S1_x_at	CA631315	Putative uncharacterized protein Sb09g001060 n=1 Tax=Sorghum bicolor	-2,281

Ta.14454.1.S1_s_at	BJ215196	Os09g0433800 protein n=3 Tax=Oryza sativa RepID=Q69PH9_ORYSJ	-2,293
Ta.5563.1.S1_at	CA643873	Putative uncharacterized protein OSJNBa0027N19.12 n=2 Tax=Oryza sativa	-2,341
Ta.6934.1.A1_a_at	CK209084	UPI0000D8C4F1 related cluster n=1 Tax=Danio rerio RepID=UPI0000D8C4F1	-2,385
Ta.2636.1.S1_x_at	CA605844	Putative uncharacterized protein Sb09g024850 n=2 Tax=Andropogoneae	-2,397
Ta.28847.1.S1_at	CA740446	Os01g0795100 protein n=2 Tax=Oryza sativa Japonica Group RepID=Q8S1I0_ORYSJ	-2,416
Ta.26213.1.S1_at	CD452838	Putative uncharacterized protein Sb06g022610 n=2 Tax=Andropogoneae	-2,418
Ta.23219.1.A1_x_at	CA737258	wpi2s.pk001.n10 wpi2s Triticum aestivum cDNA clone wpi2s.pk001.n10 5' end	-2,422
Ta.22954.3.S1_at	CA632583	Triticum aestivum cultivar Renan clone BAC 930H14, complete sequence	-2,441
TaAffx.84069.1.S1_at	CA645540	Os03g0859100 protein n=2 Tax=Oryza sativa RepID=Q84M86_ORYSJ	-2,501
Ta.24294.1.A1_at	BQ172428	Triticum aestivum cDNA clone WHE2064_H07_O14.	-2,693
Ta.1037.1.S1_at	CA638909	Os01g0731100 protein n=4 Tax=Oryza sativa RepID=Q94EA4_ORYSJ	-2,700
TaAffx.16307.1.S1_at	BQ800749	Hordeum vulgare subsp. vulgare cDNA clone: FLbaf143f08, mRNA sequence	-2,716
Ta.3304.1.S1_at	BJ243222	Putative uncharacterized protein Sb05g001550 n=1 Tax=Sorghum bicolor	-2,804
Ta.4455.1.A1_at	BJ253690	Os01g0842500 protein n=2 Tax=Oryza sativa RepID=Q8S2A8_ORYSJ	-2,810
Ta.6965.1.S1_at	BJ313225	Wh_yf Triticum aestivum cDNA clone whyf4e06 5'.	-2,845
Ta.3813.1.A1_at	BQ238416	Putative uncharacterized protein Sb05g026610 n=1 Tax=Sorghum bicolor	-3,158
Ta.29640.1.S1_x_at	CK205489	Putative uncharacterized protein n=2 Tax=Zea mays RepID=B7ZYD6_MAIZE	-3,213
Ta.10151.1.S1_at	BJ234600	Putative uncharacterized protein Sb09g016470 n=2 Tax=Sorghum bicolor	-3,503
Ta.556.1.S1_x_at	CA671780	Os05g0153300 protein n=3 Tax=Oryza sativa RepID=Q65XP3_ORYSJ	-3,531
Ta.556.1.S1_at	CA671780	Os05g0153300 protein n=3 Tax=Oryza sativa RepID=Q65XP3_ORYSJ	-3,849
Ta.28480.1.S1_s_at	CD452788	Os03g0111200 protein n=3 Tax=Oryza sativa RepID=Q10SU0_ORYSJ	-4,001
Ta.11506.1.S1_a_at	CK215703	Os07g0617500 protein n=2 Tax=Oryza sativa RepID=Q8GS08_ORYSJ	-4,843
TaAffx.85922.1.S1_x_at	CA618396	Putative uncharacterized protein (Fragment) n=5 Tax=Triticeae RepID=Q9M540_AGRCR	-11,074
Ta.7832.1.S1_at	CA646083	Putative uncharacterized protein Sb01g007930 n=2 Tax=Poaceae RepID=C5X0B2_SORBI	-114,258
TaAffx.85922.1.S1_s_at	CA618396	Putative uncharacterized protein Sb01g007930 n=2 Tax=Poaceae RepID=C5X0B2_SORBI	-114,866

Legend : According to Affymetrix Gene Chip® wheat genome array of the 168 Unknown genes or proteins of Probe sets IDs differentially regulated complemented with BLAST results showing the Genbank accession number, UniProt and NCBI description presented in decreasing order of differential expression of ≥ 2 fold and ≤ -2 fold cut off and p-value $p \leq 0,05$.

Table S5. 7 Primers used for this study, their references or GenBank Accession Number

Primers	**GenBank Accession Number or**
RT-PCR study	
5'-AATATACACGCGCCACATCA-3'*TmCir-FW*	
5'-CAAGATGTCACAGCAGCACA-3 *TmCir-RV*	
5'-AGGCCTTCTGACAACTGGAA-3'*TmUnG-FW*	**CA646083**
5'-TGTGTGCCACAGCAGTACAA-3' *TmUnG-RV*	
5'-GGAGCAGAGGCAACTTTTTG-3'*TmPHYC-FW*	**AY244514**
5'-ATTCCACCGTGTTCATCTCC-3' *TmPHYC-RV*	
5'-TCGTGGAGAAGCAGAAGGC-3' *VRN1-BAC 81655L*	**Shimada et al 2009**
5'-GTTGATGTGGCTCACCATCC-3' *VRN1-BAC 82017R*	
5'-TAAGAAGGAAGGGGGAATGG-3' *WFT-FW*	**Shimada et al 2009**
5'-GAGGGCTCTCGTAGCACATC-3' *WFT-RV*	
RT-PCR and qRT-PCR studies	
5'-ATGCCTAGTAAGCGCGAGTCAT-3' *18S_RNA-FW*	**Diallo et al 2010**
5'-ACGGGCGGTGTGTACAAAG-3'*18S_RNA-RV*	
qRT-PCR study	
5'-CAGGCCGGTCGATCTATACTA-3' *WFT-F4*	**Shimada et al 2009**
5'-TCCTGTTCCCGAAGGTCA-3' *WFT-R4*	
5'-GGAGAGGTCACTGCAGGAGGA-3' *WAP1-545L*	**Shimada et al 2009**
5'-GCCGCTGGATGAATGCTG-3' *WAP1-698R*	
5'-GAAGGTCAGAAGATGTGGAGAGTCAAC-3' *TaGI-3L*	**Shimada et al 2009**
5'-GGCAGCGGATGGTAGGTGATAG-3' *TaGI-3R*	
5'-ATGTCACCCCAGGTTATTGG-3'*qTmCYS-F*	**AY244511**
5'-TTTTCGGTCAACCCAGACAG-3' *qTmCYS-R*	
5'-ATCCACGACGCAGCATTAC-3'*qTaFlav-F*	**DQ208192**
5'-GTTGTGCTCAAGCAAAAACAAG-3' *qTaFlav-R*	
5'-GCCCATCTGGTGTGTGTTC-3',*qTaOMT-F*	**BT009571**
5'-CCATTGACGAGTAAGGCACTG-3', *qTaOMT-R*	
5'-TGTGTCTGTGCTGATGCAGA-3'*qTmPHYC-F*	**AY244514**
5'-GGCATGCTAAACTGTTGTGTG-3' *qTmPHYC-R*	

5'-CCCTCACTGTTTATTTCCCTGTG-3'*qTmCir-F*	**AK334473**
5'-GAATGAGACGGCAGACACT-3' *qTmCir-R*	
5'-CCCAACAGCATCTCCATTTG-3' *qTdLOX-B1-F*	**AK333416**
5'-TAGTTCAGCCACACACACACAC-3' *qTdLOX-B1-R*	

Figure S1: Phenotype of maintained vegetative phase (*mvp*) plants and control plants (WT). After 3 months of growing at 20°C under LD conditions pictures were taken to show the root architecture (bottom face middle panel and side face right panel) and the difference of development between *mvp* and WT plants (left panel).

Figure S2: Effect of methyl jasmonate treatment on plant development in wheat wild type cv einkorn. a) After 3 weeks of germination at 20°C under long-day photoperiod (LD), **b**) non-treated spring wheat plants (cv Manitou) were kept under LD conditions at 20°C and **c**) treated plants were sprayed with 150 µM of methyl jasmonate everyday for 2 weeks under the same growth conditions. Pictures were taken for 21 day-old control plants immediately after MeJA treatment: 2 weeks of control and treated plants and **d)** two weeks after the end of MeJA treatment for control and treated plants.

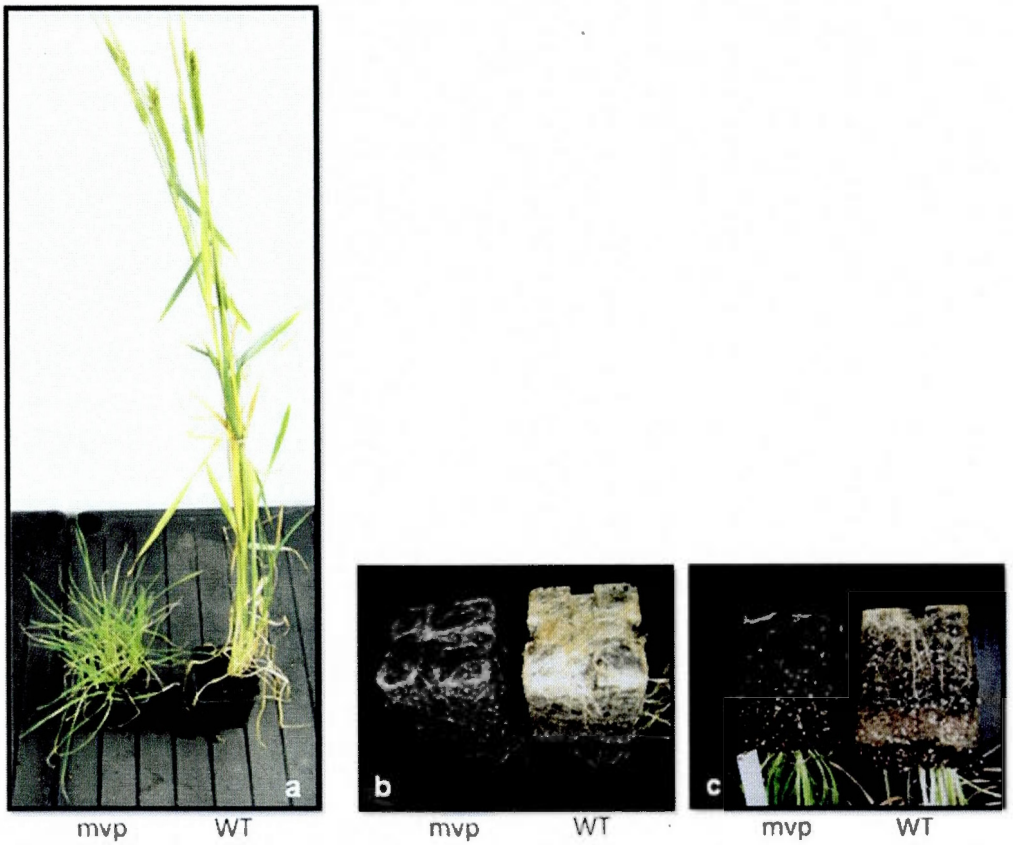

Figure S1

Figure S5. 1 Phenotype of maintained vegetative phase (*mvp*) plants and control plants (WT)

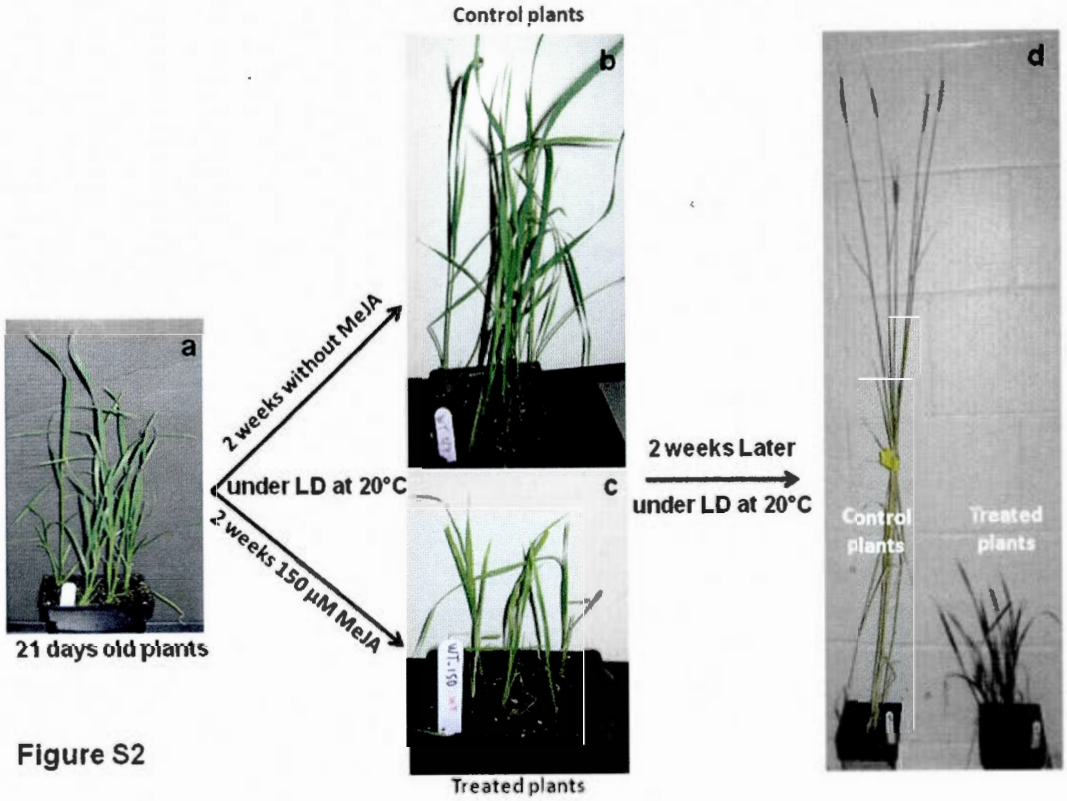

Figure S5. 2 Effect of methyl jasmonate treatment on plant development in wheat wild type cv einkorn

CHAPITRE VI

DISCUSSION GÉNÉRALE

Le but de ce chapitre est de discuter des résultats les plus importants des travaux présentés dans cette de thèse. Cette discussion porte sur le processus de régulation de la floraison en réponse à la vernalisation et en présence de l'hormone methyl jasmonate. Ce processus de floraison est régulé via le gène majeur *VRN1* et en interaction avec *VRN2*, *VRT2* et *VRN3,* trois autres gènes impliqués dans la floraison. Afin de comprendre la régulation et les fonctions de ces gènes, une approche de génomique (Microarray) combinant des outils bioinformatiques et une approche de génétique moléculaire (expression, répression, suppression et surexpression géniques et épigénétique) ont été utilisées.

6.1 LA RELATION ENTRE *VRN1, VRN2 ET VRN3* ET L'EXIGENCE DE VERNALISATION ET LA TOLERANCE AU GEL

Une exposition prolongée au froid active l'expression de *VRN1* (Danyluk *et al.*, 2003 ; Murai *et al.*, 2003 ; Yan *et al.*, 2003). Cependant les mécanismes impliqués restent peu connus. Des expériences de transformation transitoire sur les feuilles de tabac (*Nicotiana benthamiana L.*) suggèrent que la fusion d'une région de -1kbp du promoteur du gène *VRN1* à un gène rapporteur (Green Fluorescent Protein) peut servir de médiateur de l'induction du froid sur l'activité du gène rapporteur (Kane *et al.*, 2007). Ceci suggère que l'activation de *VRN1* par le froid est contrôlée par des éléments de régulation dans le promoteur.

Une analyse des modifications des histones au locus de *VRN1* et *FT1 (VRN3)* chez les plants de blé vernalisés a montré que la chromatine des gènes *VRN1* et *FT1* possède une modification des histones spécifiques qui est généralement associée à un état de chromatine active induit par la transcription d'une modification d'un marqueur d'activation: histone-3-lysine-4-triméthylation (H3K4Me3). L'expression de cette modification est trouvée au niveau de leur promoteur contenant des éléments *cis* associés à une régulation épigénétique (Diallo et al en processus de publication).

Tandis que chez les plants d'orge qui n'ont pas été vernalisés, l'analyse des modifications des histones au locus de *VRN1* a montré que la chromatine du gène *VRN1* contient une modification des histones associée à un état de chromatine inactive induit par la transcription d'une modification d'un marqueur de répression: histone-3-lysine-27-triméthylation (H3K27Me3). L'induction de l'expression de cette modification est trouvée dans le premier intron de *VRN1* et aussi au niveau du promoteur (Oliver *et al.*, 2009). Une analyse des séquences des promoteurs *VRN1*, *VRN2* et *VRN3* chez les céréales d'hiver qui requirent la vernalisation pour fleurir contiennent des éléments de régulation *cis* conservés associés à la vernalisation et à la régulation épigénétique (Diallo et al en processus de publication). L'état de la chromatine (l'emballage de l'ADN en association avec les histones) semble être un important déterminant de l'activité de *VRN1* et *FT1* chez le blé (Diallo et al en processus de publication) et de *VRN1* chez l'orge (Oliver *et al.*, 2009). Ces modifications de la chromatine peuvent être héritées par des divisions cellulaires, de sorte que ces modifications épigénétiques, et les changements associés à l'état de la chromatine, pourraient permettre à *VRN1* et *FT1* de rester actifs durant et après vernalisation et ainsi réguler la floraison. Cela pourrait contribuer à une mémoire cellulaire de vernalisation chez les céréales.

VRN2 est un répresseur de la floraison qui est exprimé dans les feuilles en périodes de longues journées (Diallo *et al.*, 2010 ; Dubcovsky *et al.*, 2006) et il semble probable que *VRN2* retarde la floraison par la suppression de l'induction de *VRN3* en périodes de jours longs (Hemming *et al.*, 2008). *VRN1* régule négativement *VRN2* (Loukoianov *et al.*, 2005), de sorte que la régulation négative de *VRN2* par *VRN1* fournit un mécanisme pour permettre l'induction de *VRN3* en périodes de jours longs chez les plantes vernalisées (Hemming *et al.*, 2008). On ne sait pas si la protéine VRN1 interagit directement avec le gène *VRN2*. Des plantes transgéniques d'*Arabidopsis thaliana* surexprimant le gène *TaVRN-B2* du blé hexaploïde (*Triticum eastivum*) ont montré un retard de floraison en comparaison des plantes contrôle

(Diallo *et al.*, 2010) même si ce gène n'a pas d'orthologue connu chez *Arabidopsis*. Ces résultats démontrent l'association de l'expression du gène *VRN2* avec le retard de floraison des plantes transgéniques suggérant ainsi la possibilité que VRN2 soit un répresseur universel.

Des résultats obtenus sur des mesures de tolérance au gel et l'expression des gènes de résistance au froid chez des lignées de plantes transgéniques d'*Arabidposis thaliana* surexprimant le gène *VRN2* du blé, un des répresseurs de *VRN1* donc de la floraison, ont montré une meilleure résistance et tolérance au gel (Diallo *et al.*, 2010). Ces résultats supportent l'importance du gène *VRN2* dans la régulation de la tolérance au gel. Quel que soit le mécanisme, cette relation entre l'activité de *VRN1 et VRN2* et la tolérance au gel est importante et a des implications pour les sélectionneurs de céréales, puisque la modification du besoin de vernalisation peut également affecter la survie durant l'hiver.

6.2 L'ANALYSE TRANSCRIPTOMIQUE DU MUTANT MVP ET L'IMPACT DE L'ABSENCE DE *VRN1*

L'analyse transcriptomique des parties aériennes des plantes du mutant *mvp,* montre que 368 gènes sont différentiellement exprimés en comparaison des plantes de type sauvage du blé diploïde *Triticum monococcum*. Globalement, cette analyse a permis d'identifier six classes de gènes à partir des 368 gènes (1 à 6). Les classes sont : la classe 1 (les gènes associés au stress biotique) représentée par 45 gènes (12,2% des gènes régulés de manière différente), la classe 2 (des facteurs de transcription qui comptent 26 gènes soit 9% des gènes), la classe 3 (les gènes associés au métabolisme du sucre qui comptent 17 gènes soit 4,6% des gènes), la classe 4 (les gènes associés au stress oxydatif qui comptent 21 gènes soit 5,7% des gènes), la classe 5 (les gènes régulés diversement, ils représentent 24,7% des gènes) et des gènes de fonction inconnu qui représentent la classe 6 soit 45,5% des gènes. Ces

résultats indiquent que la méthode de transformation utilisée pour produire le mutant *mvp* n'est pas spécifique car elle a introduit de nombreux changements dans les plantes du mutant *mvp* et a provoqué la suppression des gènes *TmAGLG1*, *TmCYS*, *TmPHYC* et *TmUnG* en plus de *VRN1*. Parmi les gènes qui sont régulés de manière différente, 105 sont surexprimés et 263 sont réprimés. La majorité de ceux fortement régulés à la hausse codent pour des protéines associées au stress biotique tel que la flavanone hydroxylase, chitinase, thaumatine, lipoxygénase, endochinase, dehydrine, defensine vasculaire. Des études ont montré que les gènes qui sont régulés à la hausse chez la plante suite à un traitement au MeJA comprennent ceux qui sont impliqués dans la biosynthèse du jasmonate, le métabolisme secondaire, la formation de la paroi cellulaire, et ceux codant pour des protéines de protection et de défense contre les nématodes (Cheong et Choi, 2003 ; Nahar *et al.*, 2011). Il est connu que les jasmonates activent les mécanismes de défense des plantes en réponse aux blessures par les insectes, divers agents pathogènes et les stress environnementaux, comme la sécheresse, la basse température et la salinité (Wasternack et Hause, 2002). L'ensemble de ces résultats et plus particulièrement la surexpression des gènes qui codent pour les enzymes flavanone hydroxylase et lipoxygénase suggèrent une régulation à la hausse de la biosynthèse des jasmonates dans les plantes du mutant *mvp*. Ces observations nous ont conduits à quantifier les jasmonates (JA et MeJA) chez les plantes du mutant *mvp* et chez les plantes de type sauvage. Nos résultats ont révélé que les plantes du mutant *mvp* accumulent environ six fois plus de méthyl jasmonate (MeJA) par rapport aux plantes de type sauvage suggérant ainsi que le MeJA pourrait être associé à la floraison chez le blé.

6.3 L'ACCUMULATION DE FT1 PERMETTRAIT D'ÉLIMINER LE MEJA, CE QUI FAVORISE LA FLORAISON

L'automne et l'hiver sont caractérisés par une diminution graduelle de la durée de la photopériode et la présence de stress comme le froid. Il est connu que les plantes induisent l'accumulation des jasmonates afin de se protéger contre le stress biotique et abiotique (Rohwer et Erwin, 2008). Dans le cas du blé d'hiver, les plantes acquièrent la compétence à fleurir avant le stress dévastateur (le gel) d'hiver. Durant cette période les plantes devraient avoir un haut niveau d'accumulation des jasmonates afin d'activer leurs mécanismes de défense. Ceci suggère que les jasmonates protègent la plante contre les divers stress pour qu'elle développe sa compétence à fleurir. Mais pour fleurir, la plante a besoin de baisser le niveau de l'accumulation de l'hormone MeJA et cela se fera par l'augmentation de la durée de la photopériode au printemps et en été.

Cette diminution de MeJA serait associée à l'induction de *PHYC* et *FT1* car l'accumulation de MeJA induit la répression de *PHYC* et *FT1* selon nos résultats. FT1 (VRN3) est une protéine qui agit comme une hormone, elle se déplace dans la plante (*florigen*) (Kardailsky *et al.*, 1999 ; Kobayashi *et al.*, 1999) et pourrait agir comme hormone antagoniste de MeJA. Le contenu en MeJA chez les plantes du mutant *mvp* est six fois plus élevée que celui des plantes de type sauvage. Ce résultat d'accumulation de MeJA dans les plantes du mutant *mvp* maintenues en phase végétative, suggère que l'expression du gène *VRN1*, le régulateur principal du développement de la transition à la phase reproductive est réprimée en présence de MeJA. En d'autres termes, il est possible que le produit du gène *VRN1* soit un régulateur négatif de l'hormone MeJA dans les plantes sauvages (première hypothèse).

PHYC étant un régulateur majeur de *FT1* qui est induit par la longueur de la photopériode (au printemps et en été), c'est ce qui permettra à la plante de fleurir. Donc chez le mutant *mvp*, la délétion du gène *PHYC* entrainerait la régulation à la baisse de *FT1* ce qui se traduirait par un retard de floraison même si la plante serait compétente à fleurir. Ceci nous permet de proposer une deuxième hypothèse qui est qu'en période de longue photopériode (printemps et été), l'accumulation de la protéine FT1 induirait à la baisse le contenu en MeJA, cette situation permettront à la plante de fleurir. Cela suggère qu'il y aurait une relation de cause à effet entre la protéine FT1 et l'hormone MeJA par exemple FT1 pourrait être répresseur de l'expression de *Flavanone hydroxylase* et/ou *Lipoxygénase*.

Afin, de dissocier les phénomènes de la compétence à fleurir et de la floraison ainsi que le rôle de MeJA dans ces deux phénomènes, des plants de blé de printemps cv Manitou âgés de 21 jours (plantes compétentes à fleurir) cultivés en conditions de jours longs ont été traités avec 150 µM de MeJA. Suite au traitement, on a analysé la floraison chez les plants contrôles (non traités) et les plants traités en calculant le pourcentage de plantes qui ont fleuri, le nombre de feuilles à la floraison et en mesurant le niveau d'expression des gènes majeurs associés à la floraison (*VRN1*, *FT1* et *PHYC*). Nos résultats ont montré clairement un retard de floraison chez les plantes traitées comparativement aux plantes témoins. Ce qui était le plus remarquable, c'est le niveau de répression de l'expression des gènes associés à la floraison chez les plantes traitées comparativement aux plantes non traitées particulièrement la répression du gène *FT1*. Ces résultats semblent favoriser la deuxième hypothèse qui stipule une relation possible entre FT1 et MeJA.

Un des domaines de recherche intéressant à poursuivre serait de valider cette possible relation entre FT1 et MeJA en mesurant le contenu de MeJA avant, pendant et après l'accumulation de FT1.

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CONCLUSION

Pour satisfaire les besoins alimentaires de la population humaine mondiale d'ici 2050 (estimée à 9,1 milliards selon la FAO), d'une façon soutenable, il est important d'améliorer les cultures et les stratégies agricoles, d'augmenter la productibilité agricole et de la survie des plantes face aux irrégularités des saisons et des perturbations dues au réchauffement de notre planète. Les produits agricoles du blé, du riz, de l'orge, du maïs et du seigle et leurs dérivés forment la base fondamentale de l'alimentation humaine et animale. Pour mieux agir et pour augmenter le rendement absolu tout en offrant la sécurité et la qualité des produits alimentaires, il est essentiel de comprendre et de pouvoir manipuler les traits quantitatifs et qualitatifs (QTLs) d'intérêt agronomique. La génomique des plantes pourra adresser ces défis. *VRN1*, *VRN2* et *VRN3* s'affichent comme candidats majeurs pour l'amélioration d'espèces essentielles pour l'alimentation humaine et animale telles que le blé, et autres plantes céréalières.

Avec l'avènement de nouvelles technologies en génétique et l'utilisation d'approches et d'outils moléculaires comme la fusion de gènes rapporteurs, des puces à ADN et la mutagenèse ciblée, les scientifiques sont en mesure d'identifier les gènes conférant la dominance ou la récessivité de caractères qui régulent la floraison. Cette approche génomique, au point de vue pratique et économique (temps et énergie), est plus intéressante et est à privilégier. Elle permet de caractériser de nombreux gènes de régulation de la réponse aux stresses environnementaux et de développer des marqueurs moléculaires associés ou responsables des effets des déterminants génétiques. À cet égard, les céréales comme le blé et l'orge sont des modèles génétiques qui s'avèrent être très utiles, car ils sont transformables. Il y a aussi de nombreuses populations de lignées, y compris les lignées recombinantes et doubles haploïdes qui peuvent être utilisées pour identifier des régulateurs potentiels de *VRN1*, *VRN2* et *VRN3*. Un des domaines essentiels à poursuivre les recherches est de comprendre comment les plantes ressentent le froid et comment les durées croissantes d'exposition au froid augmentent l'expression de *VRN1* et de *VRN3* et diminuent

l'expression de *VRN2*. Une meilleure compréhension de la régulation et les fonctions moléculaires de *VRN1* de *VRN2* et de *VRN3* entrainera des implications importantes dans les programmes de sélection des céréales. Il sera un jour possible de prédire avec précision combien d'allèles différents de *VRN1, VRN2* et de *VRN3* vont influencer le comportement des fleurs dans différents fonds génétiques ou environnementaux.

En conséquence cela permettra de mettre en place des stratégies de sélection céréalière sur mesure. Pour ces raisons, les études sur l'amélioration des connaissances sur les mécanismes moléculaires de régulation de l'acclimatation au froid, de la réponse à la vernalisation, de l'induction de la floraison par la vernalisation et par la photopériode chez les céréales sont reconnues comme étant des domaines importants de recherche en cours. Parce que les connaissances acquises chez ces céréales tempérées seront également utiles pour les autres graminées, y compris une gamme de graminées économiquement importantes comme le riz et le maïs.

Les conclusions principales de l'ensemble de ces travaux présentés dans le cadre de cette thèse de doctorat sont :

1. L'expression de *VRN2* induit le retard de floraison chez d'autres espèces en conférant une meilleure tolérance au gel.
2. L'expression de *VRN1* et *FT1* induisent la transition florale et la floraison respectivement, leur expression est régulée en partie de façon épigénétique en réponse vernalisation.
3. Le retard de floraison induit par le methyl-jasmonate est associé à la répression de *FT1* et de *VRN1*.

Enfin, ces travaux ont pu mettre en évidence le caractère multigénique de la vernalisation et son impact sur la tolérance au froid.

En perspectives :

Il faudrait élargir la recherche sur la régulation de la floraison en réponse à la vernalisation chez le blé en ciblant d'autres types modifications d'histones (l'acétylation; la phosphorylation et l'ubiquitination...), la méthylation de l'ADN et les ARNs non-codant.

Continuer l'investigation sur l'implication du MeJA dans la régulation de la floraison.

Améliorer les connaissances de la façon dont les plantes ressentent la température et la photopériode en relation avec la régulation des fonctions moléculaires de *VRN1*, *VRN2*, *FT1* et MeJA sur la régulation de la floraison.

Les connaissances acquises sur ces céréales tempérées seront également utiles pour d'autres graminées, y compris une gamme de graminées économiquement importants tels que le riz et le maïs.

Toutes ces connaissances se traduiront par d'importantes implications dans les programmes de sélection de céréales résistantes aux changements climatiques.

Autre contribution

Identification of genes and pathways associated with aluminum stress and tolerance using transcriptome profiling of wheat near-isogenic lines

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Contribution:

J'ai effectué une partie des expériences (extraction et analyse de la qualité d'ARN, RT-PCR et qRT-PCR) et leurs analyses. J'ai participé à la préparation des figures et j'ai effectué une lecture critique du manuscrit.

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